Substituted Isoquinolines and Quinazolines as Potential Antiinflammatory Agents. Synthesis and Biological Evaluation of Inhibitors of Tumor Necrosis Factor α

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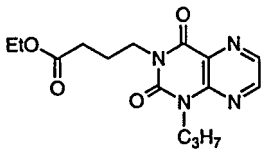
Received October 16, 1998

A series of isoquinolin-1-ones and quinazolin-4-ones and related derivatives were prepared and evaluated for their ability to inhibit tumor necrosis factor α (TNFα) production in human peripheral blood monocytes stimulated with bacterial lipopolysaccharide (LPS). In an effort to optimize the TNFα inhibitory activity, a homologous series of N-alkanoic acid esters was prepared. Several electrophilic and nucleophilic substitutions were also carried out. Alkanoic acid esters of four carbons were found to be optimum for activity in both the isoquinoline and quinazoline series. Ring substituents such as fluoro, bromo, nitro, acetyl, and aminomethyl on the isoquinoline ring resulted in a significant loss of activity. Likewise, similar groups on the quinazoline ring also reduced inhibitory activity. However, the 6- and 7-aminoquinazoline derivatives, **75** and **76**, were potent inhibitors, with IC_{50} values in the TNFα in vitro assay of approximately 5 μM for each. An in vivo mouse model of pulmonary inflammation was then used to evaluate promising candidate compounds identified in the primary in vitro assay. Compound **75** was selected for further study in this inhalation model, and was found to reduce the level of TNFα in brochoalveolar lavage fluid of LPS-treated mice by about 50% that of control mice. Thus, compounds such as **75**, which can effectively inhibit proinflammatory cytokines such as TNFα in clinically relevant animal models of inflammation and fibrosis, may have potential as new antiinflammatory agents. Finally, a quinazoline derivative suitable to serve as a photoaffinity radiolabeled compound was prepared to help identify the putative cellular target(s) for these TNFα inhibitors.

Introduction

The modulation of the biosynthesis or action of proinflammatory cytokines (PICs) such as tumor necrosis factor α (TNFα) and interleukin 1 (IL-1) has become an important strategy for pharmacological intervention in a variety of inflammatory and fibrotic disease states. Indeed, TNFα and IL-1, among other cytokines, are believed to be involved in the pathogenesis of such diverse human chronic diseases as rheumatoid arthritis,[1] psoriasis,[2] inflammatory bowel disease,[3] chronic bronchitis, and cystic fibrosis.[4] Thus, inhibition of these PICs may provide the basis for effective therapeutic treatment of these inflammatory disorders. Reported strategies for inhibition of these cytokines have included receptor antagonism, soluble receptor, neutralizing monoclonal antibodies, antiinflammatory cytokines, and cytokine synthesis inhibitors. The latter strategy has received considerable attention recently with regard to antiinflammatory drug research since this approach is most amenable to small molecule design. The low molecular weight PIC biosynthesis inhibitors encompass such diverse agents as the glucocorticoids, which inhibit transcription of PICs, the cyclic AMP phosphodiesterase (PDE) inhibitors, which elevate cAMP levels in cells, the cytokine-suppressive antiinflammatory drugs (CSAIDS), which inhibit stress-activated protein kinases such as p38 kinase, protease inhibitors, which block the processing of TNF and IL-1 precursors, inhibitors of nuclear factor kappa B activation, and various agents whose molecular targets remain undefined, such as gold salts and penicillamine.

Recently, we reported a series of pentoxifylline metabolite analogues, the most active of which were shown to be potent inhibitors of TNFα production in human peripheral blood monocytes stimulated with bacterial lipopolysaccharide (LPS).[5] A structure−activity comparison of these xanthine-like compounds in four different ring systems revealed that the pteridine system was the most promising, providing good TNF inhibitory activity without concomitant PDE inhibition or cytotoxicity. The most active derivative in the pteridine system was found to be the disubstituted pteridinedione **1** which inhibited production of TNFα at an IC_{50} of less than 5 μM in monocytes and was also active in an in vivo LPS-induced leukopenia model in mice.



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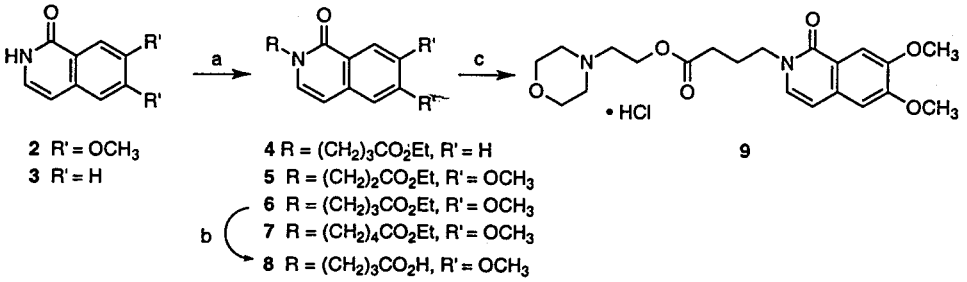
In an effort to obtain more potent TNFα inhibitors, a collection of commercially available heterocycles was acquired, each having a 6-6 membered fused ring

10.1021/jm9805900 CCC: $18.00
Published on Web 08/27/1999

Scheme 1[a]



[a] Reagents: (a) $Br(CH_2)_nCo_2Et$, NaH, DMF; (b) 1 N NaOH, reflux; (c) 1. $SOCl_2$, CH_2Cl_2: 2. 4-(2-hydroxyethyl)morpholine, CH_2Cl_2, 3. HCl.

system containing one nitrogen atom or two nitrogen atoms in the same ring. The compounds of the collection were evaluated without modification in the in vitro TNFα assay system in primary human monocytes using an ELISA detection method. The initial results revealed an active member of the isoquinoline series, 6,7-dimethoxy-1(2*H*)-isoquinolinone (**2**), which inhibited TNFα production at an IC_{50} of 50 μM. To optimize the activity in this series, a strategy similar to that which had been used for the above-mentioned pteridinediones was employed, wherein a homologous series of *N*-alkanoic acid esters was prepared. In addition, several electrophilic and nucleophilic substitutions were carried out. These approaches were subsequently extended to the quinazoline ring system. An in vivo mouse model of pulmonary inflammation was then used to evaluate promising candidate compounds identified in the primary in vitro assay. Finally, a quinazoline derivative suitable to serve as a photoaffinity radiolabeled compound was prepared in order to facilitate the identification of the putative cellular target(s) for these TNFα inhibitors.

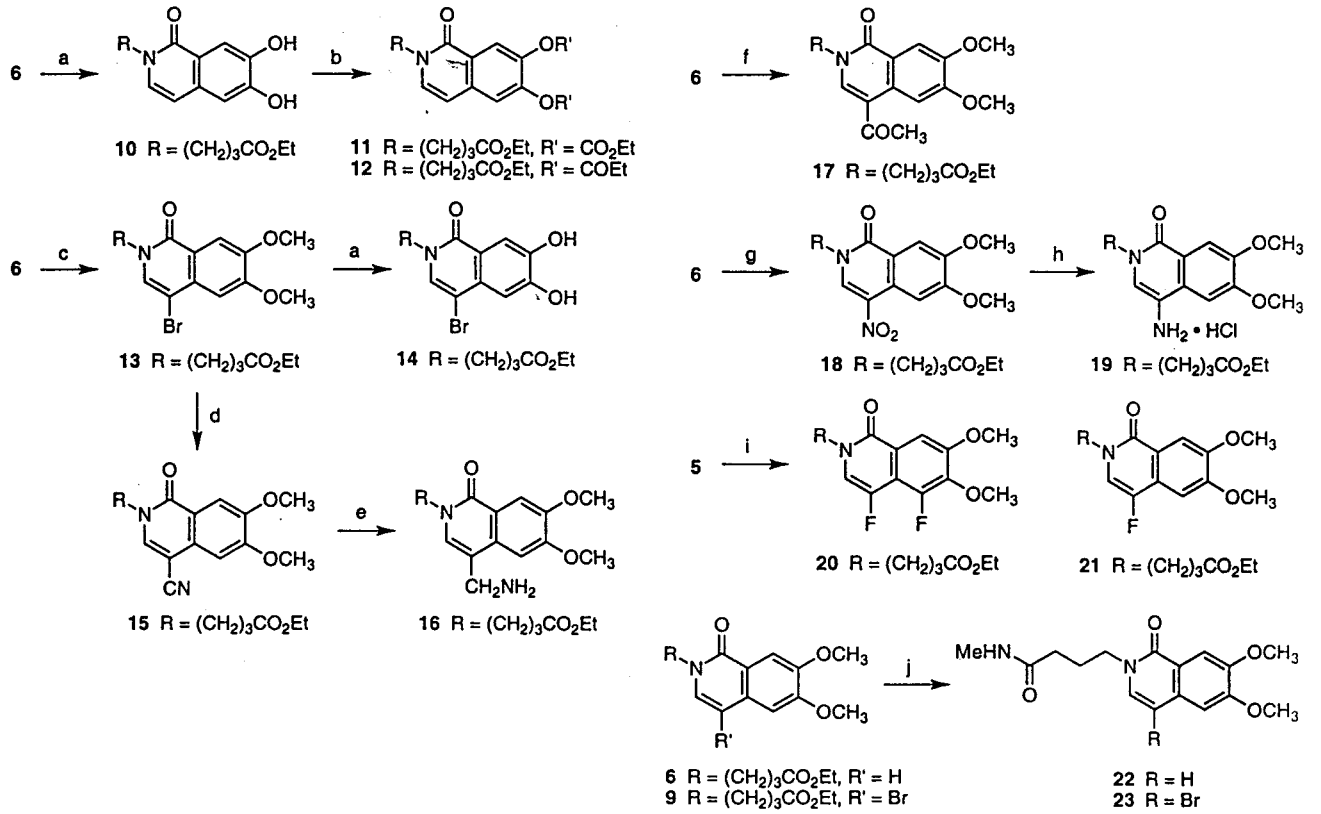
Chemistry

Isoquinolinones. In the isoquinolinone series, 6,7-dimethoxy-1(2*H*)-isoquinolinone (**2**) and its unsubstituted derivative isocarbostyril (**3**) served as convenient commercially available starting materials and required only a simple alkylation with appropriate alkyl halides. Thus, treatment of **2** or **3** with the appropriate alkyl bromide in DMF at 70 °C in the presence of sodium hydride or potassium carbonate produced the corresponding *N*-alkylated products, **4**−**7** (Scheme 1), but the yields using sodium hydride as base were much higher than those using potassium carbonate. These alkanoic ester products, however, were only sparingly soluble in water. To improve the water solubility, 2-morpholinoethyl ester **9** was prepared by reflux of ethyl ester **6** in 1 N HCl, followed by treatment of the resulting carboxylic acid **8** with $SOCl_2$ in CH_2Cl_2 and then 4-(2-hydroxyethyl)morpholine. Demethylation of **6** by treatment with BBr_3 in CH_2Cl_2 yielded the dihydroxy derivative **10** (Scheme 2). Compound **10** was converted to its dicarbonate (**11**) and diacetate (**12**) esters, respectively. Bromination of **6** by treatment with bromine in acetic acid produced the 4-bromo derivative **13**, as confirmed by its ^{1}H NMR spectrum. It has been reported[6] that electrophilic aromatic substitutions, including halogenation, acylation, and nitration, of N-alkylated isocarbostyril yield exclusively 4-substituted products. The dihydroxy derivative **14** was produced by demethylation of **13** using the same conditions as described above to obtain compound **10**. Nucleophilic substitution of the 4-bromo substituent of **13** with a cyano group was carried out by treatment with NaCN in *N*-methyl-2-pyrrolidone at high temperature to give the 4-cyano derivative **15**, which was then reduced by hydrogenation with Raney Ni as catalyst in saturated methanolic ammonia to produce the corresponding aminomethyl derivative **16**. Treatment of compound **6** with acetic anhydride in the presence of sulfuric acid gave the 4-acetyl derivative **17**. The 4-nitro derivative **18** was prepared by reaction of compound **6** with nitric acid and acetic anhydride, and hydrogenation of **18** in ethanol with palladium on activated carbon as catalyst produced the corresponding amino derivative, which was immediately converted to its hydrochloride salt **19** since the free base was subject to rapid decomposition. Treatment of compound **6** with xenon difluoride in CH_2Cl_2 gave a mixture of the 4,5-difluoroisoquinolinone **20**, the 4-fluoroisoquinolinone **21**, and recovered starting material. The structures of both compounds **20** and **21** were confirmed by ^{1}H NMR spectra. Thus, for the difluoro compound **20**, the most downfield resonance at δ 7.69 is assigned to H-8, which is split by F-5. Another doublet resonance at δ 7.56 is for H-3, which is split by F-4. There are three resonances in the aromatic region for the monofluoro compound **21**; two singlets at δ 7.80 and δ 7.09 for H-8 and H-5, respectively, and a doublet at 7.00 for H-3, which is split by F-4. Both esters **6** and **9** were converted to the corresponding amides **22** and **23**, respectively, by heating them in a sealed vessel with 40% CH_3NH_2 in H_2O.

1-Substituted Isoquinolines. In this series, we began with the known compound, 1-chloro-6,7-dimethoxyisoquinoline (**24,** Scheme 3), which was prepared by treatment of compound **2**[7] with refluxing $POCl_3$. However, this chloro derivative failed to undergo nucleophilic substitution by aliphatic or aromatic amines under a variety of conditions. Therefore, compound **24** was converted to its trifluoroacetic acid salt **25** in order to decrease the electron density on the aromatic ring and thereby facilitate nucleophilic substitutions. Subsequent reaction of **25** with primary amines was then easily carried out. Thus, reflux of compound **25** with an excess of the appropriate amine in 2-methyl-2-propanol gave the corresponding amine derivatives, **26**−**31**.

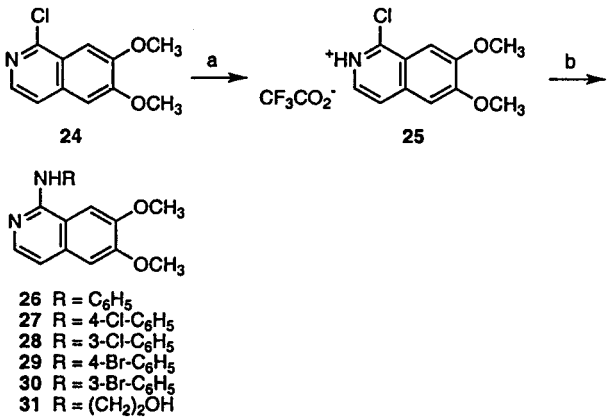
Quinazolinones. In the quinazolinone series, three groups of compounds were prepared: the 2-substituted 6,7-dimethoxy-, the 5-substituted 6,7-dimethoxy-, and various 5-, 6-, and 7-substituted quinazolinones. For the 2-substituted 6,7-dimethoxyquinazolinones, we started

Scheme 2[a]



[a] Reagents: (a) BBr_3, CH_2Cl_2; (b) EtOCOCl, pyridine, or Ac_2O, pyridine; (c) Br_2, AcOH; (d) NaCN, 1-methyl-2-pyrrolidinone; (e) H_2, Raney Ni, EtOH; (f) Ac_2O, H_2SO_4, reflux; (g) HNO_3, Ac_2O, AcOH; (h) 1. H_2, Pd/C, EtOH; 2. HCl; (i) XeF_2, CH_2Cl_2; (j) 40% H_2NMe.

Scheme 3[a]

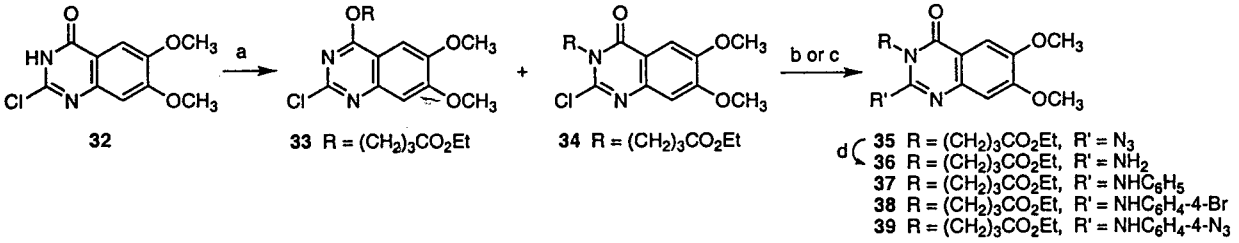


[a] Reagents: (a) TFA, 2-propanol; (b) RNH_2, *t*-BuOH.

with the versatile intermediate 2-chloro-6,7-dimethoxy-4(3*H*)-quinazolinone (**32**), which was prepared from 6,7-dimethoxy-2,4(1*H*,3*H*)-quinazolinedione[8] by treatment with $POCl_3$ followed by selective hydrolysis of the 4-chloro function using 1 *N* NaOH at room temperature for 5 h. Alkylation of **32** by the general alkylation procedure for quinazolinones (method E) gave a mixture of two products (Scheme 4), O-alkylated compound **33** (14%) and N-alkylated compound **34** (66%). These isomers were readily distinguished in the ^1H NMR since the chemical shift for the 4-CH_2 of **33** (δ 4.62), attached to oxygen, occurs further downfield than that for N-alkylated compound **34** (δ 4.35). The 2-azido derivative **35** was prepared by treatment of **34** with sodium azide in DMF. As shown in Scheme 5, the azide **35** can exist in equilibrium with the tetrazole ring form, with the azide form being favored in strongly acidic medium such as TFA.[9] Catalytic reduction of the azido group using a palladium catalyst in EtOH and TFA readily provided the 2-amino derivative **36**. The substituted aromatic amines **37**−**39** were prepared by nucleophilic substitution of the 2-chloro function of **35** with the appropriate anilines in BuOH.

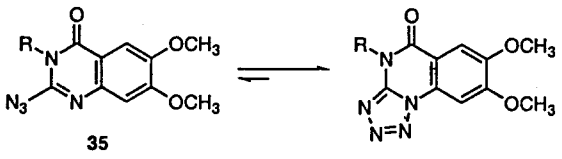
4(3*H*)-Quinazolinone (**40**), unsubstituted at the 2, 6, and 7 positions, was prepared by heating isatoic anhydride with formamidine hydrochloride at 200 °C[10] and then was alkylated at N^3 with ethyl 4-bromobutyrate by the procedure described in method E to provide **42** (Scheme 6). The corresponding 6,7-dimethoxyquinazolinone was similarly prepared by using 4,5-dimethoxyanthranilic acid with formamidine hydrochloride to form the known compound **41**,[11] and it was alkylated according to method E with haloalkanoic acid esters of three, four, and five carbons to give the corresponding N^3-alkylated quinazolinone derivatives, **43**-**45**. Demethylation of **44** to the dihydroxy derivative **46** was carried out by treatment with $AlCl_3$ in the presence of ethanethiol.[12] Attempts at nitration of the N-alkylated compound **44** with nitric acid and acetic anhydride resulted in no reaction at room temperature and hydrolysis of the ethyl ester at higher temperatures. Therefore, the nitration was carried out on nonalkylated compound **41** with potassium nitrate in sulfuric acid to produce the 5-nitro derivative **47**. Moreover, for the nitration of 6-substituted quinazolines,[13] it has been observed that formation of the 5-nitroquinazoline product is favored over that of the 8-nitro isomer.[14−17] The NMR data of the

Scheme 4[a]

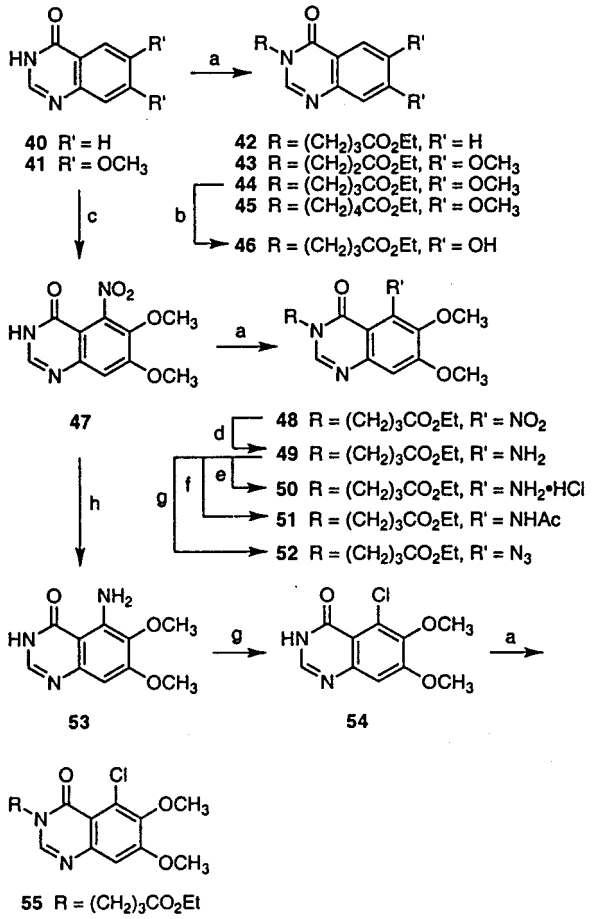


[a] Reagents: (a) $Br(CH_2)_3CO_2Et$, K_2CO_3, DMF; (b) NaN_3, wet DMF; (c) amine, BuOH; (d) H_2, Pd/C, EtOH, TFA.

Scheme 5



Scheme 6[a]



[a] Reagents: (a) $Br(CH_2)_nCO_2Et$, K_2CO_3, DMF; (b) $AlCl_3$, EtSH; (c) KNO_3, H_2SO_4; (d) H_2, Pd/C, EtOH; (e) HCl; (f) Ac_2O, pyridine; (g) 1. $NaNO_2$, 4 N HCl; 2. NaN_3, or CuCl; (h) H_2, Pd/C, AcOH, MeOH.

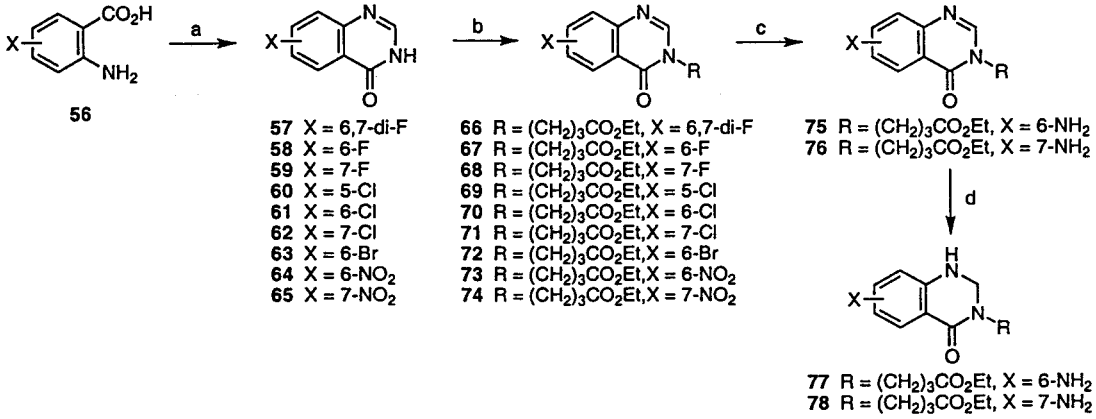
product are also consistent with the calculated data for the 5-nitro derivative. Subsequent alkylation of **47** by the general procedure gave compound **48**, which was reduced to the 5-amino derivative **49** by catalytic hydrogenation in EtOH. To increase water solubility, amino derivative **49** was converted to its hydrochloride salt **50**. Treatment of **49** with acetic anhydride in pyridine provided the corresponding 5-acetamide derivative **51**. Interestingly, the acylation of **49** was notably slower than that of normal aromatic amines, probably due to the amino group being located between the methoxy and carbonyl groups. The 5-azido derivative was prepared by diazotization of compound **49** with $NaNO_2$in 4 N HCl, followed by treatment with NaN_3. Because the side chain ester of **49** could be hydrolyzed under Sandmeyer reaction conditions, the 5-chloro derivative **55** was obtained by hydrogenation of 5-nitro **47** to its amino derivative **53**, conversion of **53** to the 5-chloro derivative **54** by the Sandmeyer reaction, and finally alkylation of **54** by method E.

For the 5-, 6-, and 7-monosubstituted quinazolinones, N-alkylated derivatives **66**−**74** were prepared by heating the mixture of appropriate substituted anthranilic acids and formamidine hydrochloride at 200 °C, followed by alkylation of compounds **57**−**65** using method E (Scheme 7). Hydrogenation of 6-nitro and 7-nitro derivatives, **73** and **74**, by using Pd/C as catalyst in EtOAc for 30 min, gave the corresponding 6- and 7-amino derivatives, **75** and **76**, respectively. However, extending the reaction time of hydrogenation of **73** and **74** to 5 h provided the respective 6- and 7-amino-1,2-dihydro derivatives **77** and **78**.

Finally, a series of 2-substituted O-alkylated quinazolines was prepared to observe any structure−activity differences in the O- versus N-alkylated isomers. Treatment of 2-chloro-6,7-dimethoxy-4(3*H*)-quinazolinone (**32**) with an appropriate amine in BuOH at elevated temperatures provided the 2-substituted amino derivatives **79**−**81** (Scheme 8). The O-alkylated compounds **82**−**84** were then obtained in good yield by using method E. Interestingly, the major products of this alkylation were O-alkylated isomers, and only trace amounts of the corresponding N-alkylated derivatives were produced. The steric effects of the NMe_2 or NHAr at the 2-position of the quinazolinones are likely responsible for the observed predominant formation of the O- versus the N-alkylation product. The structures of **82**−**84** were confirmed by their ^1H NMR spectra.

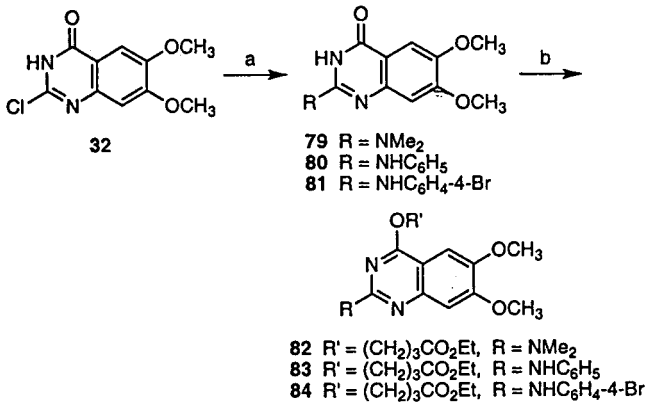
Photoaffinity Radiolabeled Derivative. The use of photoaffinity labels to elucidate the target of small molecule binding has been a valuable technique[18] for gaining information of biological interest. A compound having some activity in the TNFα in vitro assay and containing both a photoactivatable group as well as a radioisotope seemed most suitable for our purposes. The aromatic azides have been used extensively as photoreactive functional groups which produce a highly reactive nitrene species when activated by light and form a covalent bond with nearby groups at binding sites. Compound **35**, the 2-azidoquinazolinone, was first considered as a possible candidate for a photoactivatable ligand. However, preliminary studies showed that when

Scheme 7[a]



[a] Reagents: (a) formamidine hydrochloride, 210 °Cl; (b) $Br(CH_2)_3CO_2Et$, K_2CO_3, DMF; (c) H_2, Pd/C, EtOAc, 30 min; (d) H_2, Pd/C, EtOAc, 5 h.

Scheme 8[a]



[a] Reagents: (a) amine, BuOH, heat; (b) $Br(CH_2)_3CO_2Et$, K_2CO_3, DMF.

a solution of **35** was exposed to ultraviolet light of appropriate wavelength, no nitrene formation was observed, as measured by the lack of new product formation in the presence or absence of an amino acid such as serine. This resistance to photoactivation may be due to the propensity of **35** to exist in the tetrazole form (Scheme 5) as opposed to the azido form. To avoid this possibility, the 5-azidoquinazolinone **52** was then studied. This isomer was found to readily undergo photoactivation, and therefore a dehydro derivative of **52** was prepared that was suitable for radiolabeling. Thus, compound **53** was treated with sodium azide in the presence of HCl to obtain the 5-azido derivative **85**, which was then treated with methyl 4-bromocrotonate to provide the methyl 2-butenoate ester **86** (Scheme 9). Finally, compound **86** was radiolabeled by catalytic tritiation to produce the bis-labeled material **87**.

Results and Discussion

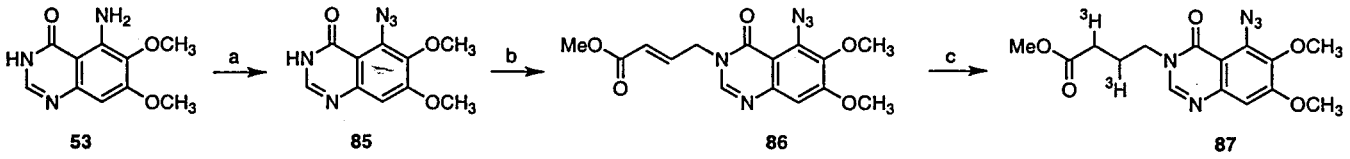
A retrospective review of the literature revealed that there are several reports of 4(3H)-quinazolinones and 1(2*H*)-isoquinolinones with antiinflammatory and related activity.[19−21] Two reports describe an in vivo rat paw edema model to investigate the aniinflammatory properties of several series of quinazolinones substituted at the 1 and 3 positions[19] and on the benzo ring of the quinazolinone.[20] Of those prepared and tested, the 3-methyl-4(3*H*)-quinazolinone and its 6-chloro derivative were found to be the most active in this system, inhibiting the paw edema by over 70% at a dose of 50 mg/kg i.p.[20] The other report details the antiallergy properties of a series of 4(3*H*)-quinazolinones substituted at 3 by propenoic acid groups determined using a passive cutaneous anaphylaxis model in rats.[11] In addition, other reports describe the activity of substituted isoquinoline-1,3-diones as cyclooxygenase 2 (COX-2) inhibitors[21] and substituted quinazoline-2,4-diones as inhibitors of nuclear factor of activated T-cells (NFAT)[22] and inhibitors of PDE IV.[23] Thus, there is considerable overlap of activities and structure−activity relationships in these heterocyclic systems. Whether or not TNFα inhibition is relevant in these reported series is not known. It is well known that PDE IV inhibition is one mechanism by which TNFα may be inhibited. [24] Also, TNFα is known to mediate COX-2 expression. [25]

Table 1 presents a summary of the physicochemical and in vitro TNFα inhibition data for the isoquinolines and quinazolines. The pteridinedione **1** was used as a positive control. Also included in the table are the results of cell toxicity experiments using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay in Jurkat cells for those compounds of most interest, i.e., those having TNFα IC_{50} values of less than 30 μM. Some structure−activity trends are evident when considering the TNF inhibition data.

Side Chain Substituents. As in the case of the previous study of substituted xanthines and related compounds,[5] a side chain length of four carbons appears to be optimum for activity in both the isoquinoline and quinazoline series. However, the quinazoline four-carbon derivative **44** is more cytotoxic than the isoquinoline of corresponding structure (**6**). Interestingly, the three-carbon side chain derivative **43** in this homologous series shows lower cytotoxicity while maintaining good TNF inhibitory activity. Esters are superior to the corresponding carboxylic acids at inhibiting TNF production, probably due to cell permeability differences (compare **6** or **9** to **8**). Corresponding amides are also inactive in this model (**22** and **23**). The water soluble 2-morpholinoethyl ester **9** is useful for administration but is slightly less active than the ethyl ester **6** and slowly undergoes hydrolysis to **8** in aqueous solution.

Ring Substituents. While the isoquinoline and quinazoline rings are isosteric, a seemingly subtle

Scheme 9[a]



[a] Reagents: (a) 1. $NaNO_2$, HCl, 2. H_2O; (b) methyl-4-bromocrotonate, K_2CO_3, DMF; (c) $^3H_2$, Pd/C.

difference of a ring nitrogen appears to exert an effect which results in at least a 10-fold loss of activity going from the unsubstituted isoquinoline **6** to the unsubstituted quinazoline **42**. In addition, the presence of the ortho dimethoxy groups are not critical to the bioactivity in the isoquinoline series (compare **4** and **6**) whereas they are important in the quinazoline series (compare **42** and **44**). However, the activity in this substituted quinazoline series is accompanied by increased toxicity as well, except for compound **43** as noted above. Moreover, cleavage of the methyl groups to form the ortho diols resulted in increased activity and toxicity in the isoquinolines (compare **6** and **10**, **13** and **14**) but decreased activity in the quinazoline series (compare **44** and **46**). In a prodrug approach, protection of the diol of **10** by esterification in an effort to decrease the toxicity, resulted in a 5-fold decrease in activity while toxicity remained about the same (compounds **11** and **12**). Substitutions at other positions on the isoquinoline ring (**13**−**23**) by groups such as bromo, aminomethyl, acetyl, nitro, and fluoro generally resulted in a significant decrease in TNFα activity. The 4-bromo and 4-cyano derivatives, **14** and **15**, respectively, however, maintained good activity but displayed increased cytotoxicity. The 1-substituted isoquinolines **27**−**31** were found to be weakly active. The unsubstituted aromatic amino derivative **26**, however, was inactive. Of the 2-substituted quinazolines, only the 2-azido derivative **35** showed significant activity with low toxicity. The 5-substituted derivatives **48**−**50** and **55** also displayed good activity with little or no toxicity at doses tested. Groups on the quinazoline other than 6,7-dimethoxy were studied and found to render the derivatives inactive. Thus, 6-fluoro, chloro, bromo, nitro, and 7-fluoro, chloro, and nitro derivatives were all devoid of TNF inhibitory activity. Interestingly, the 6-amino- and 7-aminoquinazolines **75** and **76** were good inhibitors, although **76** was also toxic. In the course of preparing these two isomeric aminoquinazolines from their respective nitro precursors, some 6- and 7-amino-1,2-dihydro products (**77** and **78**) were obtained and evaluated for activity. Both of these dihydro derivatives were found to be good inhibitors of TNFα production and to be nontoxic. Thus, partial reduction of the pyrimidine ring moiety of **75** had little effect on activity and rendered **76** much less toxic.

Finally, the 4-O-alkylated 2-substituted quinazolines, **33** and **82**−**84**, were prepared and found to have little or no TNF inhibitory activity.

In Vivo Pulmonary Inflammation Model. An in vivo mouse model of an acute lung inflammatory response was selected to determine if compounds found to have in vitro TNF inhibitory properties could be effective in a relevant in vivo setting. In this model,[26] mice are given aerosolized LPS by inhalation, which causes increased TNFα production followed by recruitment of neutrophils into the bronchoalveolar lavage fluid (BALF). Thus, LPS was given in the presence or absence of preadministered aerosolized compound and then BALF was collected 3 h later (3 h was found to be optimum[26]) and analyzed for TNFα level by an ELISA method. We selected the 6-aminoquinazoline **75** as a representative compound having in vitro TNFα inhibitory activity, low cytotoxicity, and sufficient water solubility (as the acid salt) to allow for aerosolization. Figure 1 compares the effects of pretreatment using various aerosol concentrations of **75** with that of dexamethasone as a positive control. Compound **75** was capable of reducing the level of TNFα in BALF of LPS-treated mice in a dose-dependent manner. The TNFα levels were reduced by nearly 50% that of control LPS-treated mice, and this reduction was similar to that achieved with the dexamethasone positive control.

The mechanism of action of the compounds reported here is still unknown, and efforts to elucidate their cellular target(s) are underway. The photoaffinity radiolabeled derivative **87** was successfully prepared and used in experiments, whereby lysed human THP-1 monocytic leukemia cells were incubated with **87**, photoactivated with UV light, and the resulting radiolabeled macromolecules were then examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE). Two competeable bands were clearly observed (Figure 2), one of about 16 kDa and another of about 30 kDa. Studies utilizing two-dimensional SDS−PAGE, followed by mass spectral analysis of the proteins and their digestion fragments, are presently in progress, the results of which will be reported elsewhere.

Experimental Section

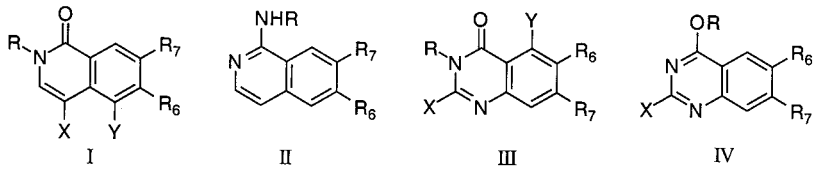
Chemistry. Melting points were obtained on a Mel-temp II capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were obtained on a Varian Unity 500 at 499.8 MHz. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane (TMS) as internal standard. Elemental analyses were performed by NuMega Resonance Labs, San Diego, CA. Thin-layer chromatography was performed on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230−400 mesh) was used for flash column chromatography.

Biological Materials. Cells. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized normal human blood by ficoll-histopaque density centrifugation. PBMC were plated in 96-well plates in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 20% autologous plasma and 2 mmol of L-glutamine at a density of 5×10^5 cells per milliliter. In some experiments, monocytes were purified from PBMC by adherence to gelatin coated flask and used at a density of 5×10^5 cells per milliliter.

Mice. Male Balb/c mice (8 weeks old) were purchased from Harlan Sprague Dawley.

Reagents. Lipopolysaccharides from *Escherichia coli* serotype 026:B6 (LPS), human TNFα, and rabbit anti-human TNFα were purchased from Sigma Chemical (St. Louis, MO). Mouse anti-human TNFα was purchased from R & D Systems

Table 1. Isoquinoline and Quinazoline Derivatives' Physical and TNFα Inhibition Data



I II III IV

compd	class	R_6 & R_7	R	X	Y	mp (°C)	formula	analyses[a]	TNFα IC_{50}[b]	MTT IC_{50}[c]
1									3	>25
4	I	H	$(CH_2)_3CO_2Et$	H	H	<40	$C_{15}H_{17}NO_3$	C, H, N	5	>50
5	I	OCH_3	$(CH_2)_2CO_2Et$	H	H	125	$C_{16}H_{19}NO_5$	C, H, N	50	
6	I	OCH_3	$(CH_2)_3CO_2Et$	H	H	84−85	$C_{17}H_{21}NO_5$	C, H, N	5	>50
7	I	OCH_3	$(CH_2)_4CO_2Et$	H	H	43	$C_{18}H_{23}NO_5$	C, H, N	21	22
8	I	OCH_3	$(CH_2)_3CO_2H$	H	H	157−159	$C_{15}H_{17}NO_5$	C, H, N	>100	
9	I	OCH_3	$(CH_2)_3CO_2M$[d]	H	H	116−118	$C_{21}H_{28}N_2O_6$·HCl	C, H, N[e]	10	>100
10	I	OH	$(CH_2)_3CO_2Et$	H	H	115−116	$C_{15}H_{17}NO_5$	C, H, N	2	47
11	I	OCO_2Et	$(CH_2)_3CO_2Et$	H	H	<50	$C_{21}H_{25}NO_9$	C, H, N	11	40
12	I	OCOMe	$(CH_2)_3CO_2Et$	H	H	58	$C_{19}H_{21}NO_7$	C, H, N	9	40
13	I	OCH_3	$(CH_2)_3CO_2Et$	Br	H	78−80	$C_{17}H_{20}BrNO_5$	C, H, N[f]	51	
14	I	OH	$(CH_2)_3CO_2Et$	Br	H	169−172	$C_{15}H_{16}BrNO_5$	C, H, N	1	11
15	I	OCH_3	$(CH_2)_3CO_2Et$	CN	H	155−157	$C_{18}H_{20}N_2O_5$	C, H, N	5	10
16	I	OCH_3	$(CH_2)_3CO_2Et$	CH_2NH_2	H	204−205	$C_{18}H_{24}N_2O_5$	C, H, N	13	7
17	I	OCH_3	$(CH_2)_3CO_2Et$	$COCH_3$	H	110−112	$C_{19}H_{23}NO_6$	C, H, N	15	47
18	I	OCH_3	$(CH_2)_3CO_2Et$	NO_2	H	151−152	$C_{17}H_{20}N_2O_7$	C, H, N	35	
19	I	OCH_3	$(CH_2)_3CO_2Et$	NH_2·HCl	H	>132 (dec.)	$C_{17}H_{22}N_2O_5$·HCl	C, H, N[g]	37	
20	I	OCH_3	$(CH_2)_3CO_2Et$	F	F	112−115	$C_{17}H_{19}F_2NO_5$	C, H, N[h]	>100	
21	I	OCH_3	$(CH_2)_3CO_2Et$	F	H	86	$C_{17}H_{19}F_2NO_5$	C, H, N	20	
22	I	OCH_3	$(CH_2)_3CONHMe$	H	H	162−165	$C_{16}H_{20}N_2O_4$	C, H, N[i]	>100	
23	I	OCH_3	$(CH_2)_3CONHMe$	Br	H	157−160	$C_{16}H_{19}BrN_2O_4$	C, H, N[j]	>100	
26	II	OCH_3	C_6H_5			165−166	$C_{17}H_{16}N_2O_2$	C, H, N	>100	
27	II	OCH_3	4-Cl-C_6H_5			167−167	$C_{17}H_{15}ClN_2O_2$	C, H, N	35	
28	II	OCH_3	3-Cl-C_6H_5			157−158	$C_{17}H_{15}ClN_2O_2$	C, H, N	33	
29	II	OCH_3	4-Br-C_6H_5			171−173	$C_{17}H_{15}BrN_2O_2$	C, H, N	38	
30	II	OCH_3	3-Br-C_6H_5			151−152	$C_{17}H_{15}BrN_2O_2$	C, H, N	37	
31	II	OCH_3	$(CH_2)_2OH$			166−167	$C_{13}H_{16}N_2O_3$	C, H, N	55	
34	III	OCH_3	$(CH_2)_3CO_2Et$	Cl	H	103−105	$C_{16}H_{19}ClN_2O_5$	C, H, N	48	
35	III	OCH_3	$(CH_2)_3CO_2Et$	N_3	H	123−125	$C_{16}H_{19}N_5O_5$	C, H, N	14	>25
36	III	OCH_3	$(CH_2)_3CO_2Et$	NH_2	H	202−5 (dec.)	$C_{16}H_{21}N_3O_5$	C, H, N	>25	
37	III	OCH_3	$(CH_2)_3CO_2Et$	NHC_6H_5	H	135−136	$C_{22}H_{25}N_3O_5$	C, H, N	>25	
38	III	OCH_3	$(CH_2)_3CO_2Et$	NHC_6H_4-4-Br	H	197−199	$C_{22}H_{24}BrN_3O_5$	C, H, N	>50	
39	III	OCH_3	$(CH_2)_3CO_2Et$	NHC_6H_4-4-N_3	H	>145 (dec.)	$C_{22}H_{24}N_6O_5$	C, H, N	>50	
42	III	H	$(CH_2)_3CO_2Et$	H	H	40	$C_{14}H_{16}N_2O_3$	C, H, N	>25	
43	III	OCH_3	$(CH_2)_2CO_2Et$	H	H	141−142	$C_{15}H_{18}N_2O_5$	C, H, N	9	>25
44	III	OCH_3	$(CH_2)_3CO_2Et$	H	H	125−126	$C_{16}H_{20}N_2O_5$	C, H, N	3	3
45	III	OCH_3	$(CH_2)_4CO_2Et$	H	H	110−112	$C_{17}H_{22}N_2O_5$	C, H, N	6	8
46	III	OH	$(CH_2)_3CO_2Et$	H	H	195−197	$C_{14}H_{16}N_2O_5$	C, H, N	48	
48	III	OCH_3	$(CH_2)_3CO_2Et$	H	NO_2	105−106	$C_{16}H_{19}N_3O_7$	C, H, N	11	>25
49	III	OCH_3	$(CH_2)_3CO_2Et$	H	NH_2	88−89	$C_{16}H_{21}N_3O_5$	C, H, N	15	>100
50	III	OCH_3	$(CH_2)_3CO_2Et$	H	NH_2·HCl	195−197	$C_{16}H_{21}N_3O_5$·HCl	C, H, N[k]	20	>100
51	III	OCH_3	$(CH_2)_3CO_2Et$	H	NHAc	104−105	$C_{18}H_{23}N_3O_6$	C, H, N	>100	
52	III	OCH_3	$(CH_2)_3CO_2Et$	H	N_3	102−104	$C_{16}H_{19}N_5O_5$	C, H, N	>25	
55	III	OCH_3	$(CH_2)_3CO_2Et$	H	Cl	120	$C_{16}H_{19}ClN_2O_5$	C, H, N	22	89
66	III	F	$(CH_2)_3CO_2Et$	H	H	107−108	$C_{14}H_{14}F_2N_2O_3$	C, H, N	>25	
67	III	6-F, 7-H	$(CH_2)_3CO_2Et$	H	H	48	$C_{14}H_{15}FN_2O_3$	C, H, N	>25	
68	III	6-H, 7-F	$(CH_2)_3CO_2Et$	H	H	80	$C_{14}H_{15}FN_2O_3$	C, H, N	>25	
69	III	H	$(CH_2)_3CO_2Et$	H	Cl	76	$C_{14}H_{15}ClN_2O_3$	C, H, N	>25	
70	III	6-Cl, 7-H	$(CH_2)_3CO_2Et$	HG	H	60	$C_{14}H_{15}ClN_2O_3$	C, H, N	>25	
71	III	6-H, 7-Cl	$(CH_2)_3CO_2Et$	H	H	90−91	$C_{14}H_{15}ClN_2O_3$	C, H, N	>25	
72	III	6-Br, 7-H	$(CH_2)_3CO_2Et$	H	H	54−55	$C_{14}H_{15}BrN_2O_3$	C, H, N	>25	
73	III	6-NO_2, 7-H	$(CH_2)_3CO_2Et$	H	H	88	$C_{14}H_{15}N_3O_5$	C, H, N	>25	
74	III	6-H, 7-NO_2	$(CH_2)_3CO_2Et$	H	H	103−104	$C_{14}H_{15}N_3O_5$	C, H, N	>25	
75	III	6-NH_2, 7-H	$(CH_2)_3CO_2Et$	H	H	64	$C_{14}H_{17}N_3O_3$	C, H, N	4	>50
76	III	6-H, 7-NH_2	$(CH_2)_3CO_2Et$	H	H		$C_{14}H_{17}N_3O_3$	C, H, N	9	13
33	IV	OCH_3	$(CH_2)_3CO_2Et$	Cl		98−99	$C_{16}H_{19}ClN_2O_5$	C, H, N	68	
77	III	6-NH_2, dihydro	$(CH_2)_3CO_2Et$	H	H	54−56	$C_{14}H_{19}N_3O_3$	C, H, N	10	>100
78	III	7-NH_2, dihydro	$(CH_2)_3CO_2Et$	H	H	109−111	$C_{14}H_{19}N_3O_3$	C, H, N	5	>50
82	IV	OCH_3	$(CH_2)_3CO_2Et$	NMe_2		79	$C_{18}H_{25}N_3O_5$	C, H, N	>25	
83	IV	OCH_3	$(CH_2)_3CO_2Et$	NHC_6H_4-4-Br		94	$C_{22}H_{24}BrN_3O_5$	C, H, N	>25	
84	IV	OCH_3	$(CH_2)_3CO_2Et$	NHC_6H_4-4-Br		94	$C_{22}H_{24}BrN_3O_5$	C, H, N	>25	

[a] All compounds analyzed for C, H, N; results were within ±0.4% of theoretical values. [b] Concentration of compounds in μM which inhibited the production of TNFα by 50% of control. [c] Concentration of compounds in μM which inhibited the growth by 50% of control. [d] M = 2-morpholinoethyl hydrochloride. [e] C: calcd, 57.21; found, 55.19. [f] C: calcd, 51.27; found, 52.24. [g] C: calcd, 55.06; found, 51.10. [h] C: calcd, 57.46; found, 58.30. [i] N: calcd, 8.94; found, 9.53. [j] N: calcd, 7.31; found, 7.74. [k] H: calcd, 5.96; found, 5.48.

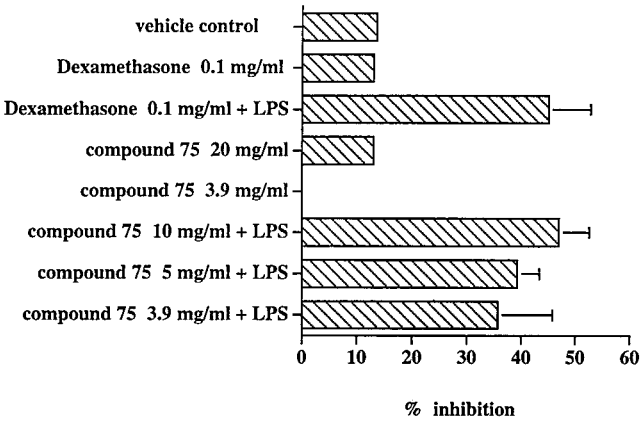


Figure 1. Effect of **75** by inhalation on TNFα production in a mouse pulmonary inflammation model. BALF from LPS-treated and nontreated mice (three animals in each group) in the presence or absence of pretreatment with compound **75** or dexamethasone (positive control) was analyzed for TNFα content by ELISA, expressed as percent inhibition of control levels. Horizontal bars represent standard error with $p < 0.05$.

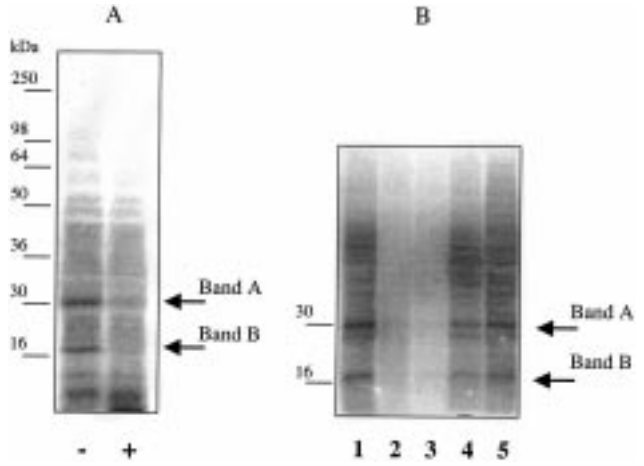


Figure 2. Photoaffinity labeling of THP-1 cytosolic extracts with **87**: (A) Autoradiogram of **87**-labeled proteins separated by SDS−14% polyacrylamide gel electrophoresis. The extract was labeled (10 min, 4 °C, 254 nm) in the presence (+) or absence (−) of a 1000-fold excess of nonlabeled photoaffinity analogue as the competing ligand. Arrows indicate position of potential target proteins. (B) Effect of preincubation with a 1000-fold excess of compounds on the **87**-labeling of cytosolic THP-1 extracts. Lane 1: **87** alone (positive control). Lane 2: nonlabeled **87**. Lane 3: **44**. Lane 4: **9**. Lane 5: **1**.

Minneapolis, MN). Peroxidase-conjugated affiniPure goat anti-rabbit IgG was purchased from Jackson ImmunoResearch Labs (West Grove, PA). Tetramethylbenzidine came from Kirkegaard and Perry (Gaithersburg, MD). Other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI).

In Vitro TNFα. Compounds in RPMI-1640 (2× concentrations) were added in equal volume to the human PBMC in 96-well microtiter plates, followed by a 60 min incubation. LPS (Salmonella, Minnesota) was then added to the cultures at a final concentration of 10 μg/mL. Eighteen hours later, 50 μL of each supernatant was collected and assayed for TNFα content by ELISA.[5]

TNFα ELISA. Human Monocytes. ELISA plates were coated overnight with 2 μg/mL mouse anti-human TNFα antibody then blocked for 2 h with 1% bovine serum albumin. Aliquots of culture (50 μL) supernatant were transferred to the plate and incubated 2 h at 37 °C and then washed. The plate was then incubated 1 h with 50 μL of 1:20000 rabbit anti-TNFα antibody and washed. Peroxidase-conjugated affiniPure goat anti-rabbit IgG antibody at 1:10000 was then added for 1 h, and the plate developed. TNFα levels were determined by extrapolation from a standard curve that was included in each assay. Samples were run in at least three separate experiments for compounds whose IC_{50} was less than 10 μM, and the variations were less than 10% among samples from healthy donors. The method for internal variability was smaller than 1%.

TNFα ELISA. Mouse BALF. A procedure similar to that described above for the human monocyte ELISA was used in the BALF model except that rat anti-mouse TNFα and biotinylated rat anti-mouse TNFα antibodies were used for the detection system.

In Vivo Pulmonary Inflammation TNFα. This model was used essentially as described by Gonçalves de Moraes et al.[26] Briefly, mice (three in each group) were pretreated with drugs either by inhalation or intraperitoneal injection. One hour later mice were treated with LPS (*E. coli*) at 0.3 mg/mL by inhalation for 10 min in an exposure chamber (Braintree Scientific, Braintree, MA). The aerosol was generated by an ultrasonic nebulizer (Ultra-Neb 99, DeVilbiss, particle size 0.5−5 μm) which was connected to an exposure chamber. Three hours later animals were sacrificed by CO_2 asphyxiation, and tracheotomy was performed. Bronchoalveolar lavage fluid was collected and centrifuged at 300*g* for 10 min. Supernatant was collected and stored at −70 °C for TNFα ELISA. The pellet was resuspended in PBS and spun in a Cytospin for 10 min at 40 speed before cells were stained with Wright-Giemsa stain (Baxter Scientific). Neutrophil differential counting versus leukocytes and TNFα level were compared to a control group and expressed as a percentage inhibition of control levels.

Photoaffinity Labeling. Human monocytic THP-1 cells were grown to a concentration of (1−2) × 10^6/mL in RPMI-1640 containing 10% fetal bovine serum. The cells were harvested, washed in PBS, and resuspended in lysing buffer (100 mM Tris pH 7.4, 40 mM NaCl, 0.5% NP-40, 1 mM EDTA, 1 mM dithiothreotol, 1 mM phenylmethylsulfonyl fluoride (PMSF)). The lysates were ultracentrifuged at 100000*g* at 4 °C for 60 min, and 5−10 μL of the supernatants (100 μg proteins) were incubated with 10 μL of PBS (pH 7.4) containing 1 μCi (1 μM) of compound **87**. In the competition experiments, the "cold" unlabeled compounds were added to the lysates 30 min before the radiolabeled compound **87**. Samples were equilibrated at 4 °C for 30 min in a 96-well plate. Wells of the plate were aligned with the axis of a UV light at a distance of 2 cm between the bottom of the wells and the lamp bulb and irradiated at 254 nm for 5−15 min at 0 °C. Sample buffer (4 × SDS) was added to the reaction mixture wells, and the proteins were separated by SDS−polyacrylamide gel electrophoresis on 14% Laemmli precast gels. The proteins were then electrotransferred to polyvinylidene fluoride (PVDF) membranes, air-dried, and exposed to autoradiography (BioMax MS) films with BioMax TransScreen-LE intensifying screens (Eastman Kodak, Rochester, NY) for 3−5 days at −80 °C. No covalent incorporation of the radiolabel occurred in the absence of UV irradiation. All of the photolabeling experiments were performed in at least three independent replicates. The films were digitized on a UMAX-Astra 1200 scanner using Adobe Photoshop, and the densities of the bands were determined by NIH IMAGE 1.59 to find the relative incorporation.

Method A: General Alkylation Procedure for Isoquinolinone Compounds 4−7. To a solution of 1(2*H*)-isoquinolinone (**3**) or 6,7-dimethoxy-1(2*H*)-isoquinolinone (**2**, 1 mmol) in 10 mL of dry DMF was added NaH (60% dispersion in mineral oil, 1.2 equiv) or K_2CO_3 (1.2 equiv). After the mixture was stirred at room temperature for 20 min, the appropriate alkyl bromide (1.2 equiv) was added, and the mixture was then stirred at 70 °C for 8−12 h. The solvent was evaporated under high vacuum, water was added to the residue, and it was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product. Crystallization of the crude product in EtOAc or flash silica gel chromatography using 0−3% MeOH/CH_2Cl_2 as eluant gave analytically pure products **4**−**7**.

Method B: General Procedure for Demethylation of Isoquinolinone Compounds 6 and 13. To a solution of an appropriate compound (**6** or **13**, 1 mmol) in 10 mL of dried CH_2Cl_2 at −78 °C was added dropwise 5 equiv of 1.0 M solution of boron tribromide in CH_2Cl_2. The reaction mixture was stirred for 1 h, during which time it was brought to room temperature, and another 2 h at room temperature. Ice−water was added, and the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−5% MeOH in CH_2Cl_2 as eluant to yield analytical pure products, **10** and **14**.

Method C: General Procedure for Transformation of Ester 6 and 9 to Amide 22 and 23. A mixture of an appropriate compound (**6** or **9**, 1 mmol) in 10 mL of methylamine (40% in water) was placed in a sealed steel reaction vessel and heated at 100 °C for 20 h. After most of the remaining methylamine and water was evaporated, 20 mL of 5% $NaHCO_3$ solution was added and the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−6% MeOH in CH_2Cl_2 as eluant to yield analytically pure products, **22** or **23**.

Method D: General Procedure for Substitution of 1-Chloro-6,7-dimethoxyisoquinoline (24) with Amines to Compounds 26−31. A solution of 1-chloro-6,7-dimethoxyisoquinoline (**24**, 1 mmol)[6] in 5 mL of 2-propanol was treated with trifluoroacetic acid (1.2 equiv). The solvent was evaporated to give trifluoroacetate **25** as a white solid. The trifluoroacetate **25** and the appropriate amine (2.2 mmol) were dissolved in 10 mL of 2-methyl-2-propanol, and the reaction mixture was brought to reflux. After reflux for 16−20 h, the mixture was cooled to room temperature, quenched with saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−2% MeOH in CH_2Cl_2 as eluant. Crystallization of the pure products from ethyl ether yielded analytically pure compounds **26−31**.

Method E: General Alkylation Procedure for Quinazolinone Compounds. A suspension of the appropriate quinazolinone compound (1 mmol), alkyl bromide (1.3 equivalent), and potassium carbonate (2 equivalent) in 10 mL of DMF was stirred at 70 °C for 8−10 h. The solvent was evaporated under high vacuum, water was added to the residue, and the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product. Crystallization of the crude product in EtOAc or flash silica gel chromatography using the appropriate solvent system yielded analytically pure compounds **33**, **34**, **42−45**, **48**, **55**, **66−74**, and **82−84**.

Method F: General Procedure for Substitution of Ethyl 4-(2-Chloro-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (34) or 2-Chloro-6,7-dimethoxy-4(3*H*)-quinazolinone (32) with Amines to Compounds 37−39 and 77−79. A solution of compound **34** or **32** and an appropriate amine (2 equiv) in 10 mL of 1-butanol was refluxed for 10−18 h. For compounds **37−39**, the solvent was evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−4% MeOH in CH_2Cl_2 as eluant to produce analytically pure compounds. For compounds **77−79**, the reaction mixture was filtered and washed with 2-propanol to yield the products which were used directly in the next step without further purification.

Method G: General Procedure for Preparation of Quinazolinone Compounds 57−65. An appropriate substituted 2-aminobenzoic acid (3.0 mmol) and formamidine hydrochloride (1.5 equiv) were ground and mixed very well and then spread around the bottom of a round-bottom flask. After heating at 210 °C for 15 min, the reaction mixture was allowed to cool to room temperature. To the mixture was added a dilute NaOH solution (0.3 N, 10 mL). The resulting solid was collected, washed with water, and dried under vacuum at 50−60 °C to yield the corresponding quinazolinone compounds, which were used directly without further purification.

Ethyl 4-(1,2-Dihydro-1-oxoisoquinolin-2-yl)butanoate (4). Compound **4** was prepared from **3** by method A using NaH as base in 75% yield as a waxy solid (269 mg): mp <40 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, 1H), 7.63 (t, 1H), 7.46−7.51 (m, 2H), 7.08 (d, 1H), 6.50 (d, 1H), 4.11 (q, 2H), 4.06 (t, 2H), 2.39 (t, 2H), 2.11 (m, 2H), 1.23 (t, 3H). Anal. $C_{15}H_{17}NO_3 \cdot 0.5H_2O$.

Ethyl 3-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)propanoate (5). Compound **5** was prepared from **2** by method A using K_2CO_3 as base to yield **4** as white solid in 32% yield (183 mg): mp 125 °C; ^{1}H NMR (DMSO-d_6) δ 7.55 (s, 1H), 7.31 (d, 1H), 7.12 (s, 1H), 6.5 (d, 1H), 4.14 (t, 2H), 4.01 (q, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 2.74 (t, 2H), 1.11 (t, 3H). Anal. $C_{16}H_{19}NO_5$.

Ethyl 4-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (6). Compound **6** was made from **2** by method A using NaH as base in 72% yield as a white solid (1.36 g): mp 84−85 °C; ^{1}H NMR (DMSO-d_6) δ 7.59 (s, 1H), 7.32 (d, 1H), 7.15 (s, 1H), 6.54 (d, 1H), 4.02 (q, 2H), 3.97 (t, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 2.32 (t, 2H), 1.93 (m, 2H), 1.51 (t, 3H). Anal. $C_{17}H_{21}NO_5$.

Ethyl 5-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)pentanoate (7). Compound **7** was made from **2** by method A using K_2CO_3 as base to yield the white solid product in 24% yield (116 mg): mp 43 °C; ^{1}H NMR (DMSO-d_6) δ 7.82 (d, 1H), 7.40 (s, 1H), 7.30 (s, 1H), 7.22 (d, 1H), 4.44 (t, 2H), 4.02 (q, 2H), 3.88 (s, 6H), 2.40 (t, 2H), 1.82 (m, 2H), 1.73 (m, 2H), 1.14 (t, 3H). Anal. $C_{18}H_{23}NO_5$.

4-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)-butanoic Acid (8). A solution of **6** (50 mg, 0.16 mmol) in 1 N NaOH (3 mL) was heated at reflux for 90 min, cooled, and neutralized with H+ ion-exchange resin. The white solid which formed was extracted into ethyl acetate, dried over Na_2SO_4, and evaporated to yield 27 mg (59%): mp 157−159 °C; ^{1}H NMR (DMSO-d_6) δ 7.59 (s, 1H), 7.32 (d, 1H), 7.15 (s, 1H), 6.54 (d, 1H), 3.97 (t, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 2.32 (t, 2H), 1.93 (m, 2H). Anal. $C_{15}H_{17}NO_5$.

2-Morpholinoethyl 4-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate, Hydrochloride (9). Compound **8** (1.0 g, 3.4 mmol) was dissolved with warming in dichloromethane (25 mL). Thionyl chloride (1 mL, 13.7 mmol) was added followed by a few drops of DMF. After a few minutes a white solid formed, assumed to be the acid chloride. The reaction mixture was evaporated to dryness. The residue was suspended in dry acetonitrile (5 mL), and morpholinoethyl alcohol (1.24 mL, 10.2 mmol) was added. The mixture was heated at 80 °C for 5 min, cooled, and absorbed onto silica gel and chromatographed using 5% MeOH−CH_2Cl_2. A small amount of 1 N HCl was added to the residue and the residue, was placed in the freezer overnight, yielding 930 mg (62%): mp 116−118 °C; ^{1}H NMR (DMSO-d_6) δ 7.77 (s, 1H), 7.01 (d, 1H), 6.84 (s, 1H), 6.40 (d, 1H), 4.18 (t, 2H), 4.05 (t, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 3.67 (t, 4H), 2.58 (t, 2H), 2.46 (t, 4H), 2.39 (t, 2H), 2.10 (m, 2)H). Anal. $C_{21}H_{28}N_2O_6 \cdot HCl$.

Ethyl 4-(1,2-Dihydro-6,7-dihydroxy-1-oxoisoquinolin-2-yl)butanoate (10). Compound **10** was prepared from **6** by using method B as needles in 76% yield (686 mg): mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 9.99 (br, 1H, OH), 8.25 (s, 1H), 6.93 (d, 1H), 6.89 (s, 1H), 6.69 (br, 1H, OH), 6.44 (d, 1H), 4.07−4.14 (m, 4H), 2.39 (t, 2H), 2.10 (m, 2H), 1.21 (t, 3H). Anal. $C_{15}H_{17}NO_5$.

Ethyl 4-[6,7-Bis(ethoxycarbonyloxy)-1,2-dihydro-1-oxoisoquinolin-2-yl]butanoate (11). To a solution of compound **10** (291 mg, 1.0 mmol) in 5 mL of dry pyridine under argon was added dropwise neat ethyl chloroformate (271 mg, 2.5 mmol). The reaction mixture was stirred at room temperature for 24 h, poured into ice−water, and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product which was purified by flash silica gel chromatography using 0−3% MeOH in CH_2Cl_2 as eluant to yield compound **11** as a waxy solid in 78% yield (339 g): mp < 50 °C; ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H), 7.46 (s, 1H), 7.10 (d, 1H), 6.45 (d, 1H), 4.34 (q, 2H), 4.33 (q, 2H), 4.12 (q, 2H), 4.04 (t,

2H), 2.37 (t, 2H), 2.09 (m, 2H), 1.38 (t, 3H), 1.379 (t, 3H), 1.24 (t, 3H). Anal. $C_{21}H_{25}NO_9$.

Ethyl 4-(6,7-Bis(acetoxy)-1,2-dihydro-1-oxoisoquinolin-2-yl)butanoate (12). To a solution of compound **10** (200 mg, 0.68 mmol) in 9 mL of dry pyridine under argon was added dropwise acetic anhydride (1 mL). The reaction mixture was stirred at room temperature for 18 h, poured into ice−water and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product. The crude product was purified by flash silica gel column chromatography using 0−3% MeOH in CH_2Cl_2 as eluant to yield compound **12** as a white solid in 81% yield (207 mg): mp 58 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (s, 1H), 7.37 (s, 1H), 7.08 (d, 1H), 6.44 (d, 1H), 4.11 (q, 2H), 4.04 (t, 2H), 2.32 (s, 3H), 2.31 (s, 3H), 2.08 (m, 2H), 1.23 (t, 3H). Anal. $C_{19}H_{21}NO_7$.

Ethyl 4-(4-Bromo-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (13). To a solution of compound **6** (2.13 g, 6.7 mmol) in 10 mL of acetic acid was added dropwise a solution of bromine (1.07 g, 6.7 mmol) in 5 mL of acetic acid. After being stirred at room temperature for 1 h, the reaction mixture was poured into ice−water and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by flash silica gel chromatography using 0−3% MeOH in CH_2Cl_2 as eluant. Crystallization of the pure product fraction from ethyl ether yielded compound **13** as needles in 61% yield (1.62 g): mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.29 (s, 1H), 7.15 (s, 1H), 4.12 (q, 2H), 4.04 (t, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 2.38 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{17}H_{20}BrNO_5$.

Ethyl 4-(4-Bromo-1,2-dihydro-6,7-dihydroxy-1-oxoisoquinolin-2-yl)butanoate (14). Compound **14** was prepared from **13** by using method B as needles in 81% yield (224 mg): mp 169−172 °C; ^{1}H NMR ($CDCl_3$) δ 9.30 (br, 1H, OH), 8.26 (s, 1H), 7.28 (s, 1H), 7.25 (s, 1H), 6.50 (br, 1H, OH), 4.14 (q, 2H), 4.07 (t, 2H), 2.41 (t, 2H), 2.11 (m, 2H), 1.25 (t, 3H). Anal. $C_{15}H_{16}BrNO_5$.

Ethyl 4-(4-Cyano-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (15). A solution of compound **13** (500 mg, 1.2 mmol) and CuCN (202 mg, 2.2 mmol) in 10 mL of *N*-methyl-2-pyrrolidone was heated at 200 °C for 2 h. To the reaction mixture was added 50 mL of water, and the mixture was filtered. The filtered material was washed with CH_2Cl_2, and the aqueous layer was extracted with CH_2Cl_2. The combined washings and extracts were dried over $MgSO_4$, the and solvent was evaporated. The residue was purified by flash chromatography using 50% EtOAc in hexane as eluant. Crystallization of the pure product fraction from EtOAc yielded compound **15** as needles in 73% yield (314 mg): mp 155−157 °C; ^{1}H NMR ($CDCl_3$) δ 7.77 (s, 1H), 7.66 (s, 1H), 7.11 (s, 1H), 4.13 (q, 2H), 4.10 (t, 2H), 4.04 (s, 3H), 4.00 (s, 3H), 2.38 (t, 2H), 2.11 (m, 2H), 1.25 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 172.61, 160.59, 154.60, 150.49, 139.00, 128.98, 119.19, 116.13, 108.08, 104.20, 90.51, 60.70, 56.40, 56.28, 49.14, 30.73, 24.30, 14.08. Anal. $C_{18}H_{20}N_2O_5$.

Ethyl 4-(4-Aminomethyl-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (16). To a solution of compound **15** (340 mg, 0.99 mmol) in 20 mL of saturated ethanolic NH_3 was added Raney Ni as catalyst, and the mixture was shaken under 25 psi hydrogen for 7 h. The reaction mixture was filtered and washed with ethanol. The filtrate was evaporated to yield an oil that was triturated with EtOAc. The analytically pure **16** was formed as a white solid in 88% yield (302 mg): mp 204−206 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.084 (s, 1H), 7.078 (s, 1H), 4.08 (q, 2H), 4.03 (t, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 2.60 (br, 2H, NH_2), 2.38 (t, 2H), 2.09 (m, 2H), 1.21 (t, 3H); Anal. $C_{18}H_{24}N_2O_5$.

Ethyl 4-(4-Acetyl-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (17). To a solution of compound **6** (150 mg, 0.47 mmol) in 8 mL of acetic anhydride was added 8 drops of concentrated sulfuric acid, and the reaction mixture was brought to reflux for 1 h. The mixture was poured into ice−water, stirred 30 min, and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$, and solvent was evaporated. The residue was purified by flash silica gel chromatography using 0−5% MeOH in CH_2Cl_2 as eluant to yield compound **17** as a yellow solid in 58% yield (98 mg): mp 110−112 °C; ^{1}H NMR ($CDCl_3$) δ 8.58 (s, 1H), 7.97 (s, 1H), 7.89 (s, 1H), 4.11−4.16 (m, 4H), 4.03 (s, 3H), 3.99 (s, 3H), 2.57 (s, 3H), 2.41 (t, 2H), 2.15 (m, 2H), 1.24 (t, 3H). Anal. $C_{19}H_{23}NO_6$.

Ethyl 4-(1,2-Dihydro-6,7-dimethoxy-4-nitro-1-oxoisoquinolin-2-yl)butanoate (18). To a solution of compound **6** (1.00 g, 3.1 mmol) in 10 mL of acetic anhydride at 0 °C was added dropwise a solution of nitric acid (3.1 mmol) in 1 mL of acetic acid. The reaction mixture was stirred at room temperature for 1 h. Ice was added to the mixture and stirred for another 20 min, and then the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$, and solvent was evaporated. The residue was purified by flash silica gel chromatography using 0−2% MeOH in CH_2Cl_2 as eluant to yield compound **18** as a pale yellow solid in 55% yield (624 mg): mp 151−152 °C; ^{1}H NMR ($CDCl_3$) δ 8.60 (s, 1H), 8.20 (s, 1H), 7.83 (s, 1H), 4.18 (t, 2H), 4.14 (q, 2H), 4.07 (s, 3H), 4.02 (s, 3H), 2.42 (t, 2H), 2.16 (m, 2H), 1.25 (t, 3H). Anal. $C_{17}H_{20}N_2O_7$.

Ethyl 4-(4-Amino-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate Hydrochloride (19). To a solution of compound **18** (100 mg, 0.27 mmol) in 5 mL of ethanol and 5 mL of EtOAc was added palladium on activated carbon (palladium content 10%, 10 mg). After the mixture was shaken under 30 psi of hydrogen for 2 h, 0.5 mL of 1 N HCl was added to the reaction mixture. The mixture was then filtered with Celite and the filter cake washed with ethanol. The filtrate was evaporated, and the residue was crystallized from 2-propanol to yield compound **19** as a white solid in 56% yield (56 mg): mp > 200 °C (dec); ^{1}H NMR (DMSO-d_6) δ 9.90 (br, 3H), 7.65 (s, 1H), 7.42 (s, 1H), 7.29 (s, 1H), 3.95−4.02 (m, 4H), 3.92 (s, 3H), 3.88 (s, 3H), 2.35 (t, 2H), 1.91 (m, 2H), 1.14 (t, 3H). Anal. $C_{17}H_{22}N_2O_5 \cdot HCl$.

Ethyl 4-(4,5-Difluoro-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanoate (20) and Ethyl 4-(1,2-Dihydro-6,7-dimethoxy-4-fluoro-1-oxoisoquinolin-2-yl)butanoate (21). To a solution of compound **5** (300 mg, 0.9 mmol) in 10 mL of dry CH_2Cl_2 at −78 °C under argon was added xenon difluoride (304 mg, 1.8 mmol). The reaction mixture was stirred overnight, and the temperature was gradually raised to room temperature. To the mixture was added 10 mL of 5% sodium bicarbonate solution, and the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by flash silica gel chromatography using 50−75% EtOAc in hexane as eluant. Difluoride compound **20** eluted first from the column and was obtained as a pale yellow solid (61 mg, 18%). The second product to elute was monofluoride compound **21**, as a pale yellow solid in 35% yield (119 mg). Finally, starting material **3** was recovered (about 20%).

Physical data for compound **20**: mp 112−115 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, 1H), 7.56 (d, 1H), 4.07−4.11 (m, 4H), 4.06 (s, 3H), 4.02 (s, 3H), 2.38 (t, 2H), 2.01 (m, 2H), 1.22 (t, 3H). Anal. $C_{17}H_{19}F_2NO_5$.

Physical data for compound **21**: mp 86 °C; ^{1}H NMR ($CDCl_3$)-δ 7.80 (s, 1H), 7.09 (s, 1H), 7.00 (d, 1H), 4.12 (q, 2H), 4.03 (t, 2H), 4.02 (s, 3H), 4.01 (s, 3H), 2.39 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{17}H_{20}FNO_5$.

***N*-Methyl 4-(1,2-Dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanamide (22).** Compound **22** was prepared from **6** using method C and was obtained as a white solid in 65% yield (45 mg): mp 162−165 °C;^{1}H NMR ($CDCl_3$) δ 7.74 (s, 1H), 7.00 (d, 1H), 6.85 (d, 1H), 6.79 (br, 1H, NH), 6.44 (s, 1H), 4.04 (t, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 2.77 (d, 3H), 2.20 (t, 2H), 2.06 (m, 2H). Anal. $C_{16}H_{20}N_2O_4 \cdot 0.5H_2O$.

***N*-Methyl 4-(4-Bromo-1,2-dihydro-6,7-dimethoxy-1-oxoisoquinolin-2-yl)butanamide (23).** Compound **23** was prepared from **9** using method C and was obtained as a white solid in 59% yield (168 mg): mp 157−160 °C; ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H), 7.32 (s, 1H), 6.54 (s, 1H), 4.05 (t, 2H), 4.03 (s, 3H), 4.01 (s, 3H), 2.78 (d, 3H), 2.22 (t, 2H), 2.09 (m, 2H). Anal. $C_{16}H_{19}BrN_2O_4$.

6,7-Dimethoxy-1-phenylaminoisoquinoline (26). Compound **26** was prepared from **25** and aniline by method D and was obtained as a white solid in 85% yield (154 mg): mp 165−166 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 1H), 7.48 (d, 2H), 7.30 (m, 2H), 7.13 (s, 1H), 7.05 (d, 1H), 6.98−7.01 (m, 2H), 6.91 (br, 1H, NH), 3.99 (s, 3H), 3.91 (s, 3H). Anal. $C_{17}H_{16}N_2O_2$.

1-(4-Chlorophenyl)amino-6,7-dimethoxyisoquinoline (27). Compound **27** was made from **25** and 4-chloroaniline as described in method D and was obtained as a white solid in 74% yield (189 mg): mp 157−158 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 1H), 7.46−7.49 (m, 2H), 7.28−7.31 (m, 2H), 7.09−7.10 (m, 2H), 7.05 (s, 1H), 6.75 (br, 1H, NH), 4.02 (s, 3H), 4.00 (s, 3H). Anal. $C_{17}H_{15}ClN_2O_2$.

1-(3-Chlorophenyl)amino-6,7-dimethoxyisoquinoline (28). Compound **28** was prepared from **25** and 3-chloroaniline by using method D and was obtained as a white solid in 90% yield (156 mg): mp 166−167 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H), 7.64 (s, 1H), 7.38 (m, 1H), 7.26 (s, 1H), 7.23 (d, 1H), 7.12 (d, 1H), 7.09 (s, 1H), 7.06 (s, 1H), 6.99 (br, 1H, NH), 3.99 (s, 3H), 3.91 (s, 3H). Anal. $C_{17}H_{15}ClN_2O_2$.

1-(4-Bromophenyl)amino-6,7-dimethoxyisoquinoline (29). Compound **29** was made from **25** and 4-bromoaniline by using method D and was obtained as a white solid in 74% yield (178 mg): mp 171−173 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 1H), 7.43 (s, 5H), 7.10 (d, 1H), 7.05 (s, 1H), 6.86 (br, 1H, NH), 4.02 (s, 3H), 3.99 (s, 3H). Anal. $C_{17}H_{15}BrN_2O_2$.

1-(3-Bromophenyl)amino-6,7-dimethoxyisoquinoline (30). Compound **30** was prepared from **25** and 3-bromoaniline by using method D and was obtained as a white solid in 80% yield (192 mg): mp 151−152 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H), 7.76 (s, 1H), 7.44 (d, 1H), 7.05−7.20 (m, 5H), 6.78 (br, 1H, NH), 4.02 (s, 3H), 3.99 (s, 3H). Anal. $C_{17}H_{15}BrN_2O_2$.

6,7-Dimethoxy-1-(2-hydroxyethyl)aminoisoquinoline (31). Compound **31** was made from **25** and ethanolamine by using method D and was obtained as a white solid in 75% yield (125 mg): mp 166−167 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (d, 1H), 7.26 (s, 2H), 6.99 (br, 1H), 6.88 (d, 1H), 5.53 (br, 1H), 4.00 (s, 6H), 3.90 (t, 2H), 3.77 (t, 2H). Anal. $C_{13}H_{16}N_2O_3$.

Ethyl 4-(2-Chloro-6,7-dimethoxyquinazol-4-yloxy)-butanoate (33) and Ethyl 2-Chloro-3,4-dihydro-6,7-dimethoxy-4-oxy-3-quinazolinebutanoate (34). Alkylation of **32** by using method E produced O-alkylated compound **33** in 14% yield (126 mg) and N-alkylated compound **34** in 66% yield (605 mg).

Physical data for compound **33**: mp 98−99 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (s, 1H), 7.20 (s, 1H), 4.62 (t, 2H), 4.13 (q, 2H), 4.01 (s, 3H), 4.00 (s, 3H), 2.52 (t, 2H), 2.24 (m, 2H), 1.23 (t, 3H). Anal. $C_{16}H_{19}ClN_2O_5$.

Physical data for compound **34**: mp 103−105 °C; ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 1H), 7.03 (s, 1H), 4.35 (t, 2H), 4.12 (q, 2H), 3.98 (s, 3H), 3.97 (s, 3H), 2.44 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{16}H_{19}ClN_2O_5$.

Ethyl 4-(2-Azido-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (35). To a solution of compound **34** (200 mg, 0.56 mmol) in 10 mL of wet DMF was added sodium azide (55 mg, 0.84 mmol), and the mixture was stirred at 100 °C for 16 h. The reaction mixture was quenched with water and extracted with EtOAc. The extracts were dried over $MgSO_4$ and evaporated to yield a crude product which was purified by flash silica gel chromatography using 35−50% EtOAc in hexane as eluant to yield compound **35** in 85% yield (172 mg): mp 123−125 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (s, 1H), 7.68 (s, 1H), 4.46 (t, 2H), 4.11 (s, 3H), 4.08 (q, 2H), 4.03 (s, 3H), 2.45 (t, 2H), 2.23 (m, 2H), 1.22 (t, 3H). Anal. $C_{16}H_{19}N_5O_5$.

Ethyl 4-(2-Amino-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (36). To a solution of compound **35** (140 mg, 0.4 mmol) in 10 mL of ethanol and 1 mL of trifluoroacetic acid was added palladium on activated carbon (palladium content 10%, 20 mg). After being shaken under 50 psi of hydrogen for 24 h, the mixture was filtered with Celite, the filter cake was washed with EtOH, and the filtrate was basified to pH 8 with 2 N NaOH. The solvent was evaporated to yield crude product which was crystalled from EtOH to yield compound **36** as white solid in 94% yield (122 mg): mp 202−205 °C; ^{1}H NMR ($CDCl_3$) δ 11.15 (br, 1H, NH), 8.15 (br, 1H, NH), 7.43 (s, 1H), 6.95 (s, 1H), 4.21 (q, 2H), 4.03 (m, 5H), 3.94 (s, 3H), 2.53 (t, 2H), 1.97 (m, 2H), 1.30 (t, 3H). Anal. $C_{16}H_{21}N_3O_5$.

Ethyl 4-(3,4-Dihydro-6,7-dimethoxy-2-phenylamino-4-oxoquinazolin-3-yl)butanoate (37). Compound **37** was made from **34** and aniline by using method F and was obtained as a white solid in 89% yield (102 mg): mp 135−136 °C; ^{1}H NMR ($CDCl_3$) δ 8.36 (br, 1H, NH), 8.86 (d, 2H), 7.49 (s, 1H), 7.39 (t, 2H), 7.11 (t, 1H), 6.90 (s, 1H), 4.27 (q, 2H), 4.19 (m, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 2.56 (t, 2H), 2.08 (m, 2H), 1.31 (t, 3H). Anal. $C_{22}H_{25}N_3O_5$.

Ethyl 2-(4-Bromophenylamino)-3,4-dihydro-6,7-dimethyoxy-4-oxoquinazolin-3-yl)butyrate (38). Compound **38** was prepared from **34** and 4-bromoaniline by using method F as white solid in 66% yield (136 mg): mp 197−199 °C; ^{1}H NMR ($CDCl_3$) δ 8.47 (br, 1H, NH), 7.79 (d, 2H), 7.47−7.49 (s and d, 3H), 6.88 (s, 1H), 4.26 (q, 2H), 4.16 (m, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 2.56 (t, 2H), 2.05 (m, 2H), 1.32 (t, 3H). Anal. $C_{22}H_{24}BrN_3O_5$.

Ethyl 4-(2-[(4-Azidophenyl)amino]-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (39). Compound **39** was made from **34** and 4-azidoaniline by using method F and was obtained as a yellow solid in 63% yield (119 mg): mp 133−135 °C (dec); ^{1}H NMR ($CDCl_3$) δ 8.46 (br, 1H, NH), 7.89 (d, 2H), 7.49 (s, 1H), 7.06 (d, 2H), 6.88 (s, 1H), 4.27 (q, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 3.92 (m, 2H), 2.56 (t, 2H), 2.06 (m, 2H), 1.32 (t, 3H). Anal. $C_{22}H_{24}N_6O_5$.

Ethyl 4-(3,4-Dihydro-4-oxoquinazolin-3-yl)butanoate (42). Compound **42** was prepared from **40** by using method E and was obtained as a white solid in 72% yield (256 mg): mp 40 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (d, 1H), 8.05 (s, 1H), 7.76 (t, 1H), 7.71 (d, 1H), 7.51 (t, 1H), 4.12 (q, 2H), 4.08 (t, 2H), 2.40 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{16}N_2O_3$.

Ethyl 3-(3,4-Dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)propanoate (43). Compound **43** was prepared from **41** by using method E and was obtained as a white solid in 82% yield (424 mg): mp 141−142 °C; ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 1H), 7.63 (s, 1H), 7.11 (s, 1H), 4.25 (t, 2H), 4.13 (q, 2H), 3.99 (s, 6H), 2.88 (t, 2H), 1.22 (t, 3H). Anal. $C_{15}H_{18}N_2O_5$.

Ethyl 4-(3,4-Dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (44). Compound **44** was made from **41** by using method E and was obtained as a white solid in 87% yield (531 mg): mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1H), 7.62 (s, 1H), 7.10 (s, 1H), 4.12 (q, 2H), 4.07 (t, 2H), 3.99 (s, 6H), 2.40 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{16}H_{20}N_2O_5$.

Ethyl 5-(3,4-Dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)pentanoate (45). Compound **45** was made from **41** by using method E and was obtained as a white solid in 61% yield (491 mg): mp 110−112 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1H), 7.62 (s, 1H), 7.09 (s, 1H), 4.11 (q, 2H), 4.02 (t, 2H), 3.99 (s, 6H), 2.35 (t, 2H), 1.84 (m, 2H), 1.71 (m, 2H), 1.23 (t, 3H). Anal. $C_{17}H_{22}N_2O_5$.

Ethyl 4-(3,4-Dihydro-6,7-dihydroxy-4-oxoquinazolin-3-yl)butanoate (46). To a solution of compound **44** (200 mg, 0.62 mmol) and ethanethiol (0.7 mL) in 10 mL of dried CH_2Cl_2 under argon was added aluminum chloride (416 mg, 3.12 mmol). After the reaction mixture was stirred at room temperature for 6 h, K_2CO_3 and water were carefully added, and the mixture was stirred for another 20 min and then extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and solvent was evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−5% MeOH in CH_2Cl_2 as eluant to yield compound **46** as white solid in 57% yield (104 mg): mp 195−197 °C; ^{1}H NMR (DMSO-d_6) δ 10.00 (br, 2H, OH), 8.08 (s, 1H), 7.38 (s, 1H), 6.92 (s, 1H), 3.97 (q, 2H), 3.91 (t, 2H), 2.30 (t, 2H), 1.90 (m, 2H), 1.11 (t, 3H). Anal. $C_{14}H_{16}N_2O_5$.

Ethyl 4-(3,4-Dihydro-6,7-dimethoxy-5-nitro-4-oxoquinazolin-3-yl)butanoate (48). To a solution of compound **41** (1.00 g, 4.8 mmol) in 15 mL of concentrated H_2SO_4 was added potassium nitrate (728 mg, 7.2 mmol) in several portions. After it was stirred at room temperature for 6 h, ice and 10 N NaOH were added to the mixture to a pH of 2−3 to

precipitate the product. The product was filtered, washed with water, and dried under vacuum to yield compound **47** as little yellow solid in 60% (724 mg): mp 261−263 °C (dec), which was used directly in next step without further purification; ^{1}H NMR (DMSO-d_6) δ 12.40 (br, 1H, NH), 8.13 (s, 1H), 7.41 (s, 1H), 4.02 (s, 3H), 3.84 (s, 3H).

Compound **48** was made from **47** by using method E and was obtained as a white solid in 74% yield (2.62 g): mp 105−106 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.21 (s, 1H), 4.12 (q, 2H), 4.03 (t, 2H), 4.02 (s, 3H), 3.95 (s, 3H), 2.37 (t, 2H), 2.08 (m, 2H), 1.25 (t, 3H). Anal. $C_{16}H_{19}N_3O_7$.

Ethyl 4-(5-Amino-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (49). To a solution of compound **48** (502 mg, 1.3 mmol) in 15 mL of ethanol was added palladium on activated carbon (palladium content 10%, 60 mg). After being shaken under 35 psi of hydrogen for 24 h, the mixture was filtered with Celite, the filter cake washed with EtOH, and the solvent was evaporated. Crystallization of the crude product from EtOAc−hexane yielded compound **49** as white solid in 89% yield (386 mg): mp 88−89 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H), 6.49 (s, 1H), 6.30 (br, 2H, NH_2), 4.12 (q, 2H), 3.97 (t, 2H), 3.92 (s, 3H), 3.82 (s, 3H), 2.38 (t, 2H), 2.08 (m, 2H), 1.24 (t, 3H). Anal. $C_{16}H_{21}N_3O_5$.

Ethyl 4-(5-Amino-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate Hydrochloride (50). To a solution of compound **49** (100 mg, 0.3 mmol) in 5 mL of 2-propanol was added 1 equiv of 1 N HCl solution. The solvent was evaporated, and ethanol was added and evaporated, repeated several times. Compound **50** was obtained as a white solid in quantitative yield: mp 195−196 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (s, 1H), 6.51 (s, 1H), 5.20 (br, 3H), 3.98 (q, 2H), 3.94 (t, 2H), 3.87 (s, 3H), 3.66 (s, 3H), 2.37 (t, 2H), 1.94 (m, 2H), 1.13 (t, 3H). Anal. $C_{16}H_{21}N_3O_5$·HCl.

Ethyl 4-(5-Acetamido-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (51). A solution of compound **49** (200 mg, 0.6 mmol) and acetic anhydride (2 mL) in 8 mL of dry pyridine was stirred at room temperature for 48 h, at which time TLC showed the reaction was complete. The solvent was evaporated, water was added, and the mixture was extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and the solvent was evaporated to yield a crude product, which was purified by flash silica gel chromatography using 0−3% MeOH in CH_2Cl_2 as eluant to yield compound **51** as white solid in 60% yield (136 mg): mp 104−105 °C; ^{1}H NMR ($CDCl_3$) δ 9.65 (br, 1H, NH), 7.88 (s, 1H), 6.93 (s, 1H), 4.08 (q, 2H), 3.94 (t, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 2.33 (t, 2H), 2.19 (s, 3H), 2.02 (m, 2H), 1.19 (t, 3H). Anal. $C_{18}H_{23}N_3O_6$.

Ethyl 4-(5-Azido-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (52). To a solution of compound **49** (450 mg, 1.3 mmol) in 10 mL of 4 N HCl at −15 °C was added dropwise a solution of sodium nitrite (120 mg, 1.74 mmol) in 2 mL of water over 20 min. After the mixture was stirred for 10 min, a solution of sodium azide (174 mg, 2.7 mmol) in 2 mL of water was added. The reaction mixture was stirred at room temperature for 1 h, quenched with water, and extracted with CH_2Cl_2. The extracts were dried over $MgSO_4$ and the solvent was evaporated to yield a crude product, which was purified by flash silica gel chromatography with 0−3% MeOH in CH_2Cl_2 as eluant. Crystallization of pure product fraction from ethyl ether yielded compound **52** as white needles in 73% yield (353 mg): mp 102−104 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1H), 6.95 (s, 1H), 4.12 (q, 2H), 4.01 (t, 2H), 3.97 (s, 3H), 3.92 (s, 3H), 2.38 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{16}H_{19}N_5O_5$.

5-Amino-6,7-dimethoxy-4(3*H*)-quinazolinone (53). To a solution of compound **47** (2.51 g, 10 mmol) in 80 mL of methanol was added palladium on activated carbon (palladium content 10%, 250 mg). After being shaken under 50 psi of hydrogen for 20 h, the mixture was filtered with Celite and the filter cake washed with EtOH. The solvent was evaporated to yield compound **52** as pale yellow solid in 85% yield (1.87 g): mp >250 °C (dec); ^{1}H NMR (DMSO-d_6) δ 11.74 (br, 1H, NH), 7.82 (s, 1H), 6.72 (br, 2H, NH_2), 6.39 (s, 1H), 3.84 (s, 3H), 3.64 (s, 3H). Anal. $C_{10}H_{11}N_3O_3$·0.25H_2O.

Ethyl 4-(5-Chloro-3,4-dihydro-6,7-dimethoxy-4-oxoquinazolin-3-yl)butanoate (55). To a solution of compound **52** (1.00 g, 4.5 mmol) in 15 mL of 6 N HCl solution at −15 °C was added dropwise a solution of sodium nitrite (407 mg, 5.9 mmol) in 2 mL of water over 20 min. After the mixture was stirred for 10 min, a cold suspension of CuCl (584 mg, 5.9 mmol) in 5 mL of 6 N HCl was added. The temperature of the reaction was gradually raised to room temperature over 1 h, and then the mixture was stirred at 60 °C for another hour. After the pH of the mixture was adjusted to 5−6 with 10 N NaOH solution, the mixture was filtered and the resulting solid washed with EtOH to produce compound **54** as pale yellow solid in 74% (804 mg), which was used directly in next step without further purification: mp >230 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.13 (br, 1H, NH), 8.01 (s, 1H), 7.16 (s, 1H), 3.94 (s, 3H), 3.75 (s, 3H).

Compound **55** was made from **54** by using method E and was obtained as a white solid in 73% yield (742 mg): mp 120 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H), 7.06 (s, 1H), 4.12 (q, 2H), 4.02 (t, 2H), 3.98 (s, 3H), 3.89 (s, 3H), 2.39 (t, 2H), 2.11 (m, 2H), 1.25 (t, 3H). Anal. $C_{16}H_{19}ClN_2O_5$.

Ethyl 4-(6,7-Difluoro-4-oxoquinazolin-3-yl)butanoate (66). Compound **57** was made from **56** by using method G and was obtained as a pale yellow solid in 84% (456 mg), mp 265−267 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.48 (br, 1H, NH), 8.13 (s, 1H), 8.02 (dd, 1H), 7.74 (dd, 1H).

Compound **66** was obtained from **57** by using method E and was obtained as a white solid in 92% (602 mg): mp 120 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (dd, 1H), 8.03 (s, 1H), 7.49 (dd, 1H), 4.13 (q, 2H), 4.07 (t, 2H), 2.40 (t, 2H), 2.11 (m, 2H), 1.25 (t, 3H). Anal. $C_{14}H_{14}F_2N_2O_3$.

Ethyl 4-(6-Fluoro-4-oxoquinazolin-3-yl)butanoate (67). Compound **58** was prepared from **56** by using method G and was obtained as a white solid in 82% (404 mg), mp 255−257 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.37 (br, 1H, NH), 8.08 (s, 1H), 7.78 (dd, 1H), 7.74 (dd, 1H), 7.69 (dd, 1H).

Compound **67** was made from **58** by using method E and was obtained as a white solid in 80% (537 mg): mp 48 °C; ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.93 (dd, 1H), 7.72 (dd, 1H), 7.47 (ddd, 1H), 4.12 (q, 2H), 4.07 (t, 2H), 2.40 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}FN_2O_3$.

Ethyl 4-(7-Fluoro-4-oxoquinazolin-3-yl)butanoate (68). Compound **59** was made from **56** by using method G and was obtained as a white solid in 62% (305 mg), mp 247−248 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.32 (br, 1H, NH), 8.17 (dd, 1H), 8.13 (s, 1H), 7.44 (dd, 1H), 7.38 (dd, 1H).

Compound **68** was prepared from **59** by using method E and was obtained as a white solid in 91% (379 mg): mp 80 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (dd, 1H), 8.05 (s, 1H), 7.35 (dd, 1H), 7.22 (dt, 1H), 4.13 (q, 2H), 4.06 (t, 2H), 2.40 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}FN_2O_3$.

Ethyl 4-(5-Chloro-4-oxoquinazolin-3-yl)butanoate (69). Compound **60** was prepared from **56** by using method G and was obtained as a brown solid in 76% (546 mg), mp 270−272 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.30 (br, 1H, NH), 8.07 (s, 1H), 7.71 (t, 1H), 7.59 (d, 1H), 7.51 (d, 1H).

Compound **69** was made from **60** by using method E as white needles in 80% (606 mg): mp 76 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H), 7.59−7.61 (m, 2H), 7.49 (dd, 1H), 4.13 (q, 2H), 4.04 (t, 2H), 2.40 (t, 2H), 2.12 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}ClN_2O_3$.

Ethyl 4-(6-Chloro-4-oxoquinazolin-3-yl)butanoate (70). Compound **61** was made from **56** by using method G and was obtained as a yellow solid in 85% (612 mg), mp 268−271 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.44 (br, 1H, NH), 8.12 (s, 1H), 8.04 (d, 1H), 7.84 (dd, 1H), 7.69 (d, 1H).

Compound **70** was obtained from **61** by using method E and was obtained as a white solid in 83% (624 mg): mp 83 °C; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 1H), 8.03 (s, 1H), 7.68 (dd, 1H), 7.65 (d, 1H), 4.12 (q, 2H), 4.06 (t, 2H), 2.39 (t, 2H), 2.11 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}ClN_2O_3$.

Ethyl 4-(7-Chloro-4-oxoquinazolin-3-yl)butanoate (71). Compound **62** was made from **56** by using method G and was

obtained as a yellow solid in 81% (584 mg), mp 249−252 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.41 (br, 1H, NH), 8.13 (s, 1H), 8.10 (d, 1H), 7.72 (m, 1H), 7.55 (m, 1H).

Compound **71** was made from **62** by using method E and was obtained as a white needles in 97% (728 mg): mp 90−91 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, 1H), 8.05 (s, 1H), 7.70 (d, 1H), 7.45 (dd, 1H), 4.12 (q, 2H), 4.06 (t, 2H), 2.40 (t, 2H), 2.11 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}ClN_2O_3$.

Ethyl 4-(6-Bromo-4-oxoquinazolin-3-yl)butanoate (72). Compound **63** was prepared from **56** by using method G as brown solid in 78% (524 mg), mp 267−269 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.44 (br, 1H, NH), 8.18 (d, 1H), 8.13 (s, 1H), 7.95 (dd, 1H), 7.61 (d, 1H).

Compound **72** was made from **63** by using method E and was obtained as a white solid in 86% (647 mg): mp 54−55 °C; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, 1H), 8.05 (s, 1H), 7.83 (dd, 1H), 7.58 (d, 1H), 4.12 (q, 2H), 4.07 (t, 2H), 2.40 (t, 2H), 2.11 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{15}BrN_2O_3$.

Ethyl 4-(6-Nitro-4-oxoquinazolin-3-yl)butanoate (73). Compound **64** was made from **56** by using method G and was obtained as a yellow solid in 81% (465 mg), mp 287−288 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.77 (br, 1H, NH), 8.80 (d, 1H), 8.54 (dd, 1H), 8.04 (s, 1H), 7.86 (d, 1H).

Compound **73** was obtained from **64** by using method E as plates in 84% (512 mg): mp 88 °C; ^{1}H NMR ($CDCl_3$) δ 9.17 (d, 1H), 8.54 (dd, 1H), 8.19 (s, 1H), 7.84 (d, 1H), 4.10−4.16 (m, 4H), 2.42 (t, 2H), 2.14 (m, 2H), 1.25 (t, 3H). Anal. $C_{14}H_{15}N_3O_5$.

Ethyl 4-(7-Nitro-4-oxoquinazolin-3-yl)butanoate (74). Compound **65** was prepared from **56** by using method G and was obtained as a brown solid in 79% (452 mg), mp 269−270 °C, which was used directly: ^{1}H NMR (DMSO-d_6) δ 12.67 (br, 1H, NH), 8.37 (s, 1H), 8.34 (d, 1H), 8.22−8.26 (m, 2H).

Compound **74** was made from **65** by using method E as plates in 78% (476 mg): mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H), 8.46 (d, 1H), 8.26 (dd, 1H), 8.16 (s, 1H), 4.12 (q, 2H), 4.11 (t, 2H), 2.42 (t, 2H), 2.14 (m, 2H), 1.25 (t, 3H). Anal. $C_{14}H_{15}N_3O_5$.

Ethyl 4-(6-Amino-4-oxoquinazolin-3-yl)butanoate (75). To a solution of compound **73** (305 mg, 1.0 mmol) in 20 mL of ethyl acetate was added palladium on activated carbon (palladium content 10%, 40 mg). After being shaken under 20 psi of hydrogen for 30 min, the mixture was filtered with Celite, the filter cake was washed with CH_2Cl_2, and the solvent was evaporated. The residue was purified by flash silica gel chromatography with 0−3% MeOH/CH_2Cl_2 as eluant to produce compound **75** as a white solid in 92% yield (345 mg): mp 64 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.53 (d, 1H), 7.46 (d, 1H), 7.10 (dd, 1H), 4.12 (q, 2H), 4.04 (t, 2H), 3.98 (br, 2H), 2.39 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{17}N_3O_3$.

Ethyl 4-(7-Amino-4-oxoquinazolin-3-yl)butanoate (76). The compound **76** was produced from **74**, by the same procedure used for compound **75**, as syrup in 79% (296 mg): ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 1H), 8.01 (s, 1H), 7.34 (d, 1H), 6.99 (dd, 1H), 6.65 (br, 2H), 4.12 (q, 2H), 4.03 (t, 2H), 2.39 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{17}N_3O_3$.

Ethyl 4-(6-Amino-1,2-dihydro-4-oxoquinazolin-3-yl)butanoate (77). To a solution of compound **73** (180 mg, 0.6 mmol) in 20 mL of ethyl acetate was added palladium on activated carbon (palladium content 10%, 40 mg). After being shaken under 20 psi of hydrogen for 3 h, the mixture was filtered with Celite, the filter cake was washed with CH_2Cl_2, and the solvent was evaporated. The residue was purified by flash silica gel chromatography with 2−4% MeOH/CH_2Cl_2 as eluant to produce compound **77** as a pale yellow solid in 76% yield (140 mg): mp 54−56 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.53 (d, 1H), 7.46 (d, 1H), 7.10 (dd, 1H), 4.12 (q, 2H), 4.04 (t, 2H), 3.98 (br, 2H), 2.39 (t, 2H), 2.10 (m, 2H), 1.24 (t, 3H). Anal. $C_{14}H_{19}N_3O_3 \cdot {}^1/_4CH_2Cl_2$.

Ethyl 4-(7-Amino-1,2-dihydro-4-oxoquinazolin-3-yl)butanoate (78). To a solution of compound **74** (250 mg, 0.82 mmol) in 20 mL of ethyl acetate was added palladium on activated carbon (palladium content 10%, 40 mg). After being shaken under 20 psi of hydrogen for 3 h, the mixture was filtered with Celite, the filter cake was washed with CH_2Cl_2, and the solvent was evaporated. The residue was purified by flash silica gel chromatography with 2−4% MeOH/CH_2Cl_2 as eluant to produce compound **78** as a white solid in 60% yield (150 mg): mp 109−111 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (s, 1H), 6.19 (dd, 1H), 5.89 (d, 1H), 4.56 (s, 2H), 4.09 (br, q, 3H), 3.90 (br, 2H), 3.50 (t, 2H), 2.38 (t, 2H), 1.92 (m, 2H), 1.21 (t, 3H). Anal. $C_{14}H_{19}N_3O_3 \cdot {}^1/_8H_2O$.

Ethyl 4-(2-Dimethylamino-6,7-dimethoxyquinazol-4-yloxy)butanoate (82). Compound **79** was made by using method F and was obtained as a white solid in 82% yield (203 mg), which was used directly without further purification: mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 10.83 (br, 1H, NH), 7.24 (s, 1H), 6.73 (s, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.04 (s, 6H).

Compound **82** was made from **79** by using method E and was obtained as a white solid in 84% yield (152 mg): mp 79 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (s, 1H), 6.80 (s, 1H), 4.55 (t, 2H), 4.12 (q, 2H), 3.98 (s, 3H), 3.94 (s, 3H), 3.24 (s, 6H), 2.52 (t, 2H), 2.21 (m, 2H), 1.22 (t, 3H). Anal. $C_{18}H_{25}N_3O_5$.

Ethyl 4-(6,7-Dimethoxy-2-phenylaminoquinazol-4-yloxy)butanoate (83). Compound **80** was made by using method F and was obtained as a white solid in 73% yield (218 mg), which was used directly without further purification: mp 258−261 °C; ^{1}H NMR (DMSO-d_6) δ 10.65 (br, 1H, NH), 8.55 (br, 1H, NH), 7.72 (d, 2H), 7.31−7.35 (m, 3H), 7.01 (m, 1H), 6.89 (s, 1H), 3.87 (s, 3H), 3.80 (s, 3H).

Compound **83** was made from **80** by using method E and was obtained as a white solid in 77% yield (159 mg): mp 87 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, 2H), 7.35 (t, 2H), 7.24 (s, 1H), 7.02−6.98 (m, 3H), 4.57 (t, 2H), 4.13 (q, 2H), 4.00 (s, 3H), 3.97 (s, 3H), 2.54 (t, 2H), 2.24 (m, 2H), 1.23 (t, 3H). Anal. $C_{22}H_{25}N_3O_5$.

Ethyl 4-[2-(4-Bromophenyl)amino-6,7-dimethoxyquinazol-4-yloxy]butanoate (84). Compound **81** was made by using method F and was obtained as a white solid in 68% yield (256 mg), which was used directly without further purification: mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 9.59 (br, 2H, NH), 7.65 (d, 2H), 7.55 (d, 2H), 7.33 (s, 1H), 6.59 (s, 1H), 3.86 (s, 3H), 3.81 (s, 3H). Anal. $C_{16}H_{14}BrN_3O_3$.

Compound **84** was made from **81** by using method E and was obtained as a white solid in 82% yield (161 mg): mp 94 °C; ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 2H), 7.43 (d, 2H), 7.24 (s, 1H), 7.03 (s, 1H), 6.96 (br, 1H, NH), 4.55 (t, 2H), 4.13 (q, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 2.54 (t, 2H), 2.23 (m, 2H), 1.23 (t, 3H). Anal. $C_{22}H_{24}BrN_3O_5$.

Methyl 4-[5-Azido-6,7-dimethoxy-4-oxoquinazolin-3-yl]-2-butenoate (86). To a solution of compound **53** (800 mg, 3.6 mmol) in concentrated HCl (7 mL) and H_2O (3 mL) in an ice−salt bath (−15 °C) were dropped a solution of $NaNO_2$ (276 mG, 4.0 mmol) in H_2O (2 mL) over 20 min and then added a solution of NaN_3 (468 mg, 7.2 mmol) in H_2O (2 mL). After the cold bath was removed, the reaction mixture was stirred at room temperature overnight, during which time a precipitate was formed. The mixture was filtered and washed with H_2O. The collected precipitate was dried to give compound **85** as white solid in 75% yield (668 mg): mp >190 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.00 (br, 1H), 8.25 (s, 1H), 7.08 (s, 1H), 3.94 (s, 3H), 3.82 (s, 3H). Without further purification, compound **85** was used in the next step.

A solution of compound **85** (200 mg, 0.8 mmol), methyl 4-bromocrotonate (85%, 202 mg, 0.96 mmol), and K_2CO_3 (276 mg, 2 mmol) in 10 mL of DMF was stirred at 70 °C for 8 h. After DMF was evaporated by high vacuum, 15 mL of H_2O was added to the residue and the mixture extracted with CH_2Cl_2. The extracts were dried over M_2SO_4 and the solvent was evaporated. The crude product was purified by silica gel chromatography using 50−100 EtOAc/hexane as eluant to give compound **86** as a white solid in 73% yield (202 mg): mp 125−127 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1H), 6.99 (dt, 1H), 6.96 (s, 1H), 5.88 (dt, 1H), 4.70 (dd, 2H), 3.99 (s, 3H), 3.93 (s, 3H), 3.72 (s, 3H). Compound **86** was submitted to New England Nulclear Products for tritiation and was catalytically reduced to provide **87**.

Acknowledgment. This work was supported in part by Grant AR44850 from the National Institutes of Health.

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JM9805900